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CYTOLOGY AND BREEDING BEHAVIOR OF SELECTED PLANTS OF POA PRATENSIS[1]

ETLAR L. NIELSEN[2]

Introduction

Poa pratensis L. is one of the most ubiquitous perennial grasses occurring in the cooler humid portions of North America. It is also common throughout northern Europe. Its widespread occurrence as a forage and turf species in these regions has provoked considerable interest in the development of new types. MÜNTZING (**26**) demonstrated that an apomictic form of reproduction occurred in this species. Its economic importance and its peculiarities in reproduction have resulted in an extensive bibliography.

Examination of the literature concerning the cytology and breeding behavior of the bluegrasses reveals that much of the information is fragmentary and inconclusive. This may be partially attributed to the fact that such researches represent for the most part studies of isolated portions of the complete life history. It appeared desirable, therefore, that the scope of the present study be broadened and that certain critical phases of the entire life cycle of the plant be examined in some detail. Although such an investigation was undertaken, numerous gaps in the data still remain. A number of these, such as the comparative developmental embryology in sexual and apomictic plants, are apparent.

Material, methods, and terminology

Four families of related plants were selected for this study. The original seed lot of *P. pratensis*, from which the progenies were developed, was received from W. H. Wright of the Dominion Department of Agriculture, Ottawa, Canada. Seedlings were established in the field in 1936. Another progeny was established in 1938 from a single panicle of a plant selected in 1937. Four plants were selected from this progeny in 1939. One of these was considered as typical of the predominant morphological type of the family and three were representative aberrant plants. Seedlings from individual panicles harvested from these four plants were established in the field in 1940.[3] In 1941, adult plants of three progenies of the twenty-four plants each exhibited diverse morphological types, whereas only two aberrant plants were in evidence in the fourth.

These four progenies are described as follows. The types indicated refer to groups of plants of similar growth and morphological characteristics. They may or may not be identical plants such as would be expected from apomictic seed formation.

70. Developed from an aberrant plant. 91.7% of the progeny were aberrant and of varying morphological types. Two plants (type

[1] Results of co-operative studies between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering and the Wisconsin Agricultural Experiment Station, Madison.

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[3] Progenies from 1936 to 1940, inclusive, were grown by Drs. F. W. Tinney, O. S. Aamodt, and H. L. Ahlgren.

I) were morphologically similar to the predominant type of No. 71. Eleven plants (type II) approached the preceding in morphology but were less vigorous. Three plants (type III) were of tussock-growth habit, medium-coarse textured, and moderately leafy. Eight plants (type VII) were of varying gross morphology, no two of which appeared similar.

71. Developed from a parental-type plant (type I). These were vigorous, moderately strong creepers, leafy, and of medium texture. Two plants (8.3%) of the progeny were aberrants (type VII). Both were of semitussock-growth habit with numerous long leaves.

72. Developed from an aberrant plant. 95.8% of the progeny were aberrants. One plant (type I) was similar to the predominant type of No. 71. Three plants (type II) were essentially similar to the preceding but less vigorous. Twenty plants (type VII) were of varying morphology, no two being closely similar.

73. Developed from an aberrant plant. 87.5% of the progeny were aberrants. Three plants (type I) were similar to the predominant type of No. 71. Nine dwarf, bunch-type plants (type IV) had dark, fine-textured leaves and purple panicles. Two nonaggressive erect plants (type V) had medium-coarse leaves, and eight (type VI), which were semidwarf, had fine, dark-green leaves and purple panicles. These were somewhat more aggressive in growth habit than those of type IV. There was also one variant plant (type VII) that did not fall into any of these classes.

The data for aberrant frequency given in the description of progenies are based upon similarity to the predominant type of progeny 71. If, however, the aberrant frequency is calculated upon the basis of the predominant type in a given progeny, the aberrant frequencies are 8.3, 58.3, 87.5, and 62.5% for progenies 70, 71, 72, and 73, respectively.

Material for embryological studies was taken at regular intervals from the time of the emergence of the tip of the panicle until caryopses were at the dough stage. This was fixed in Sax's modification of Navashin's and/or Allen's modification of Bouin's fluids. These preparations were transferred 24 hours after fixation to 70% alcohol for storage. Later random samples from each fixation were imbedded, sectioned at 12–25 μ (depending upon the age of the floret), and stained in Heidenhain's or/and Delafield's haematoxylin.[4]

Other materials for smear preparations were fixed in acetic alcohol and/or chloroform–acetic alcohol. This was transferred to 70% alcohol 48 hours later, sealed, and stored until used.

During September, 1941, 6-inch cores from each of the selected plants were transplanted into an isolation plot in soil of moderately low fertility adjacent to the site to be used for progeny tests. These were for comparison with the plants of their progenies.

Selected panicles were harvested and placed in individual envelopes for storage. During early March, 1942, all the seeds from a single panicle were planted in small bread pans filled with a friable sandy loam compost. Seedlings taken at random were transplanted to individual plant bands when they were about 2–3 inches tall. These were subsequently transplanted into spaced rows in the field in May.

TERMINOLOGY.—During the interim of years that have passed since WINKLER (42) defined apomixis, an extensive bibliography concerning this phenomenon has been developed. Confusion has arisen in the usage of the terms proposed by different workers for variations in asexual reproduction. In this paper the terminology proposed by FAGERLIND (15) and

[4] Duplicate sets of herbarium specimens were prepared from each of the plants selected for embryological study. One set was retained at Madison, Wisconsin, and the other deposited in the Herbarium of the Smithsonian Institution in Washington, D.C.

by STEBBINS (39) is followed. The terms in question are:

Apomixis.—"Apomixis is the substitution for sexual reproduction of another, asexual reproductive process that does not involve nuclear or cellular fusion (that is fertilization)" (WINKLER, 42, as given by STEBBINS, 39, p. 509).

Somatic apospory.—"The embryo-sac initial is a purely somatic cell usually of the chalazal region, but sometimes of the nucellus" (39, p. 511).

The terms "parental" and "aberrant" are used in this paper to describe morphological similarity or dissimilarity of progeny members as compared with the parent seed plant. An aberrant differs morphologically from the seed parent. A plant of a progeny that resembles its seed parent is referred to as the parental type.

Results

SEED SET, GERMINATION AND SEEDLINGS

LITERATURE REVIEW.—

ZOLLIKOFER (43) studied the establishment of plants of *Poa alpina* L. from seeds and from proliferated propagules. Generally, the vigor of plants established from seed was inferior to that of young plants from proliferations. She suggested that the formation of the few seeds in plants of this species might be of importance for the evaluation of the apogamy question in species of *Poa*.

NILSSON (31) observed differences in the "cross- and self-fertility" of biotypes of *P. pratensis*. He reported that no seed formed in emasculated florets unless pollination had been effected subsequently.

ÅKERBERG (1, 2) and MÜNTZING (27) reported on the quantitative variation in the amount of seed produced by biotypes of *Poa* species of varying morphological characters. In 1942, ÅKERBERG (5) reported differences in the amount and germinability of seeds among plants in progenies of *P. pratensis*.

SPENCER and FERGUS (37) found variations in the percentages of caryopses developed in florets in seed lots of *P. pratensis* harvested in Kentucky and Ohio. Eight types were classified on the basis of floret and ovarian development. At harvest time the most poorly developed florets contained both sterile stamens and undeveloped pistils, both of which probably had been arrested during their early stages. Intermediate stages were classified on the basis of apparently functional anthers, partial development of the ovary, completely developed but shriveled seeds, seeds with opaque endosperm, and mature seeds with hard endosperm. They found no ovarian development in 46% of all the florets of the 1941 seed crop. Seventy-two per cent of the florets had incompletely developed seeds. Of the remaining, only 22.5% had developed seeds with hard endosperm, and 5% were either of soft or of soft and hard endosperm.

EXPERIMENTAL RESULTS.—

In March, 1942, marked differences were found in the germination of the seed from panicles of different plants. Seedling emergence varied from 7 to 12 days after planting, and the number varied from two or three individuals to more than 400. Although the panicles from the different plants varied somewhat in size and in the number of florets, the variation in the number of seedlings that emerged could not be explained entirely on the basis of difference in the size of the panicles. The relative numbers of seedlings produced from a panicle of the different plants are given in table 1.

Irrespective of the progeny, generally

there was no consistent, close relation among plants classified as morphologically similar and the number or vigor of the seedlings derived from a single panicle. For illustrative purposes, plants 70–20, 70–22, 73–11, and 73–21 were classified as morphologically similar (type I), but they differ markedly in the number and vigor of seedlings developed from the seeds of individual panicles.

Seeds from panicles that had been harvested in June, 1943, were planted in September and handled similarly to those grown earlier. The results of this planting very closely paralleled those of the previous year. Of the forty-one seed lots studied, five differed somewhat in the number of seedlings produced. Four of these were parental-type plants (71–6 and 10, and 73–19 and 21). The fifth was an aberrant (71–3).

Because of the lack of exact fundamental information concerning these relative observations pertaining to the seed and seedlings from different plants, duplicate seed lots from other panicles harvested in 1943 were germinated, using regular seed laboratory technique. The data given in table 1 show that the numbers of seeds in a panicle from these selected plants of four sister progenies are highly variable. The lowest average number of seeds, eleven, was produced in a panicle of plant 70–19, and the highest average number, 213, in a panicle of plant 72–20. The isolated plants were somewhat less vigorous in 1943 than in 1941. Panicles therefore produced somewhat fewer seeds in 1943.

The germinability of the seed produced was generally high. Of the forty-four lots tested, thirty-six germinated 80% or higher. Plant 72–19 produced thirty-four seeds, of which only two germinated; and from sixteen seeds of 70–2 only two seedlings developed.

There is fair agreement between the number of seedlings developed in pans in 1942 and the number of seeds and seedlings developed from the same plants in 1943. Exceptions are evident in table 1, but the statement appears to be generally true.

From these observations concerning the relative number of seedlings produced from the seeds of individual panicles from the several plants, and from the germination studies, it appears that in most cases the number of seedlings produced is determined at some stage in the ontogenetic development of the ovules rather than in the senescence of completely developed seeds.

Progeny tests

Literature review.—

Müntzing (**26**) found aneuploid chromosome numbers of matroclinic plants of uniform progenies to be similar to those of the maternal plants of *Poa alpina* and *P. pratensis*. Rancken (**35**) confirmed these results for *P. pratensis*. Flovik (**16**) reported similar breeding behavior in *P. alpigena* (E. Fries) Lindm. type *domestica* ($2n = 84$), *P. glauca* Vahl ($2n = 70$), and *P. arctica* R. Br. ($2n = 56$).

Åkerberg (**4**) reported an average of 9.2% of aberrant plants after "isolation, free-flowering and crossing" apomictic biotypes of *P. pratensis*. Collections from wild populations produced progenies with 5.9% aberrants. Brown (**12**) found 0.1–18.2% aberrant plants in progenies from seeds produced by open pollination.

Müntzing (**27**) studied F_2 and F_3 progenies of a sexual *P. pratensis* plant and concluded that apomictic seed formation is recessive to sexual propagation. It is also recessive in hybrid, haploid, and triploid plants. The cross sexual × apomictic, in *P. alpina*, tended to give haploids, indicating apomictic tend-

TABLE 1

NUMBER OF SEEDLINGS AND PERCENTAGE GERMINATION OF SEED FROM INDIVIDUAL PANICLES AND THE SEGREGATION IN PLANT PROGENIES OF POA PRATENSIS

Type	Plant	No. seedlings from single panicle in			Progeny behavior	
		Pan*	Germinator			
			No. seeds	Germination (%)	No. plants	Aberrant (%)
		Family 70†				
I	70–20	2	163	71	21	90.5
	22	5	75	79	24	37.5
II	11	4	74	96	18	100.0
III	13	5	95	82	22	63.6
	17	4	89	87	19	63.2
	19	5	11	64	24	58.3
VII	2	5	16	13	23	39.1
	6	2	66	91	24	50.0
	8	3	39	41	21	57.1
	18	5	88	93	24	25.0
		Total and weighted average			220	56.8
		Family 71				
I	71–6	2	102	87	24	25.0
	10	2	99	97	19	100.0
	12	2	48	92	24	16.7
VII	3	2	97	97	23	26.1
	8	5	82	90	20	35.0
		Total and weighted average			110	38.2
		Family 72				
I	72–5	4	184	99	19	0.0
II	11	5	47	83	23	100.0
	12	5	27	100	20	45.0
	19	5	34	6	22	72.7
VII	1	5(2, 0)‡	82	70	2	50.0
	6	5	66	91	24	100.0
	7	5	19	89	19	100.0
	8	2	82	90	23	100.0
	9	5(2, 3)‡	102	99	4	50.0
	13	5(3, 13)‡	46	96	5	20.0
	14	1	154	97	24	8.3
	16	3	44	93	23	100.0
	17	2	109	90	23	8.7
	18	4	47	85	23	100.0
	20	5(14, 18)‡	213	97	19	73.7
	22	5(14, 4)‡	56	89	8	62.5
	23	5(8, 180)‡	160	93	20	100.0
	24	5	32	88	24	100.0
		Total and weighted average			325	71.1

* Seedling classes: 1, numerous; 2, many; 3, medium; 4, few; 5, very few.

† Segregation of plants of family 70: two type-I plants, eleven type-II plants, three type III, eight type VII. Family 71: twenty-two type I, two type VII. Family 72: one type I, three type II, twenty type VII. Family 73: three type I, nine type IV, two type V, eight type VI, one type VII.

‡ Actual number of seedlings from first and second plantings, respectively.

TABLE 1—*Continued*

Type	Plant	No. seedlings from single panicle in			Progeny behavior	
		Pan*	Germinator			
			No. seeds	Germination (%)	No. plants	Aberrant (%)
		Family 73				
I	73–11	5	83	95	24	62.5
	19	3	92	93	24	29.2
	21	2	.....	.....	24	16.7
IV	1	5	27	89	23	69.6
	4	5	.....	.....	22	22.7
	8	5	24	83	21	47.6
	14	5	64	84	18	100.0
V	2	5	172	94	24	25.0
	5	5	43	56	24	70.8
VI	15	5	.....	.....	18	44.4
	16	5	.....	.....	14	35.7
		Total and weighted average.....			236	34.0

encies toward development without fertilization. The absence of apomictic F_3 progenies of *P. pratensis* was considered definite evidence that apomictic seed formation is conditioned by more than one genetic factor. The balance of these factors may be upset by crossing or by quantitative chromosomal changes. Divergence in type within a chromosomal group was considered due to mutation.

ENGELBERT (**13**) concluded from her breeding experiments that races of *Poa arctica*, *P. alpigena*, *P. alpina*, and *P. pratensis* were "apomictic (pseudogamous)."

TINNEY and AAMODT (**41**) reported no variant plants in thirty-eight of 102 individual plant progenies of *P. pratensis*. The highest percentage of aberrants reported was 21.9. The total population averaged 1.6% variant plants.

BRITTINGHAM (**10**) analyzed 115 progenies of the same species. Four were completely uniform in type. Sixty-two had 0.1–12% aberrant plants. Of the remaining forty-eight progenies, forty-one had 13–43% and eight had 44–65.5% aberrants. There was an average of 14.8% aberrants in the total population.

MYERS (**30**) selected forty-six plants from three progenies that had been studied previously by BRITTINGHAM, who had found that they contained 3, 27, and 48% variant plants, respectively. From the breeding behavior of these forty-six plants he concluded that "variant type plants produced a higher proportion of sexual seeds than did their parents of parental type sibs."

EXPERIMENTAL RESULTS.—

Forty-four progenies from plants of families 70–73, inclusive, were established in May, 1942. They were examined for vigor and general uniformity in the fall and again in 1943. Characters

used in determining variations from the parental plant type included early vigor, color, growth habit, aggressiveness, texture of foliage, earliness of vegetative growth, early heading date, relative date of ripening, number of culms, coarseness of culms, vigor at maturity, height, panicle type, late growth habit, leafiness, and leaf length.

The data concerning aberrant frequency among the progenies of the selected plants are given in table 1. Certain significant features of these should be mentioned.

Considering first the progenies from plants classified as parental type (I), there is a wide range in percentages of aberrants (figs. 1, 2). The progeny of only one plant (72–5) was uniform. Other percentages of aberrants ranged from 17 to 100%.

The type-II plants were classified on a basis of being similar in morphology to the plants of type I but somewhat less vigorous. No plant placed in type II had more than 65% of its progeny classified as similar, and in two cases totally diverse populations were encountered.

Likewise, in any other group of plants classified as similar morphologically (types III, IV, V, and VI), diverse percentages of aberrants appear in their progenies. Type III of family 70 and type VI of family 73 are the only ones wherein reasonably comparable percentages of aberrants occurred in the progenies of the three plants classified as similar.

Two plants of type VII of family 72 produced progenies with 8–9% aberrants. Hence, two plants that reproduced primarily by apomixis appeared among the twenty morphologically diverse aberrants of the previous generation. Seven of the fourteen different aberrant plants studied produced totally diverse progenies, indicating at least frequent if not entirely sexual reproduction. From this it may be assumed that in some florets of otherwise sexual plants there is a change toward apomictic reproduction. The converse also appears true in some instances (figs. 2–7).

Plants of types IV and V of family 73 were considered as dwarfs or semidwarfs. Some plants appeared in the progenies from these dwarfs and semidwarfs that were much more vigorous and differed morphologically from their parental-type sister plants. An example of these is shown in figure 8.

From these data it appears that the morphological characteristics of plants are not necessarily related to breeding behavior, nor does there appear to be any direct relationship between seedling development and progeny behavior.

Cytology

Macrosporogenesis and embryology

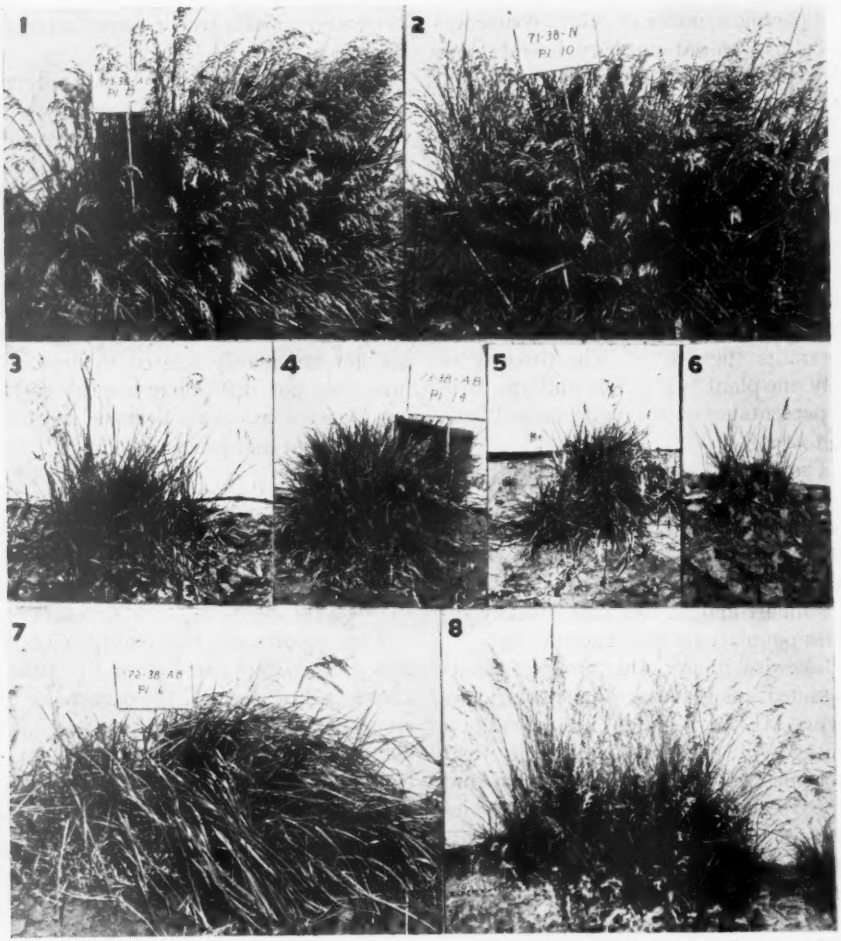
Literature review.—

Two reports on the embryology of *Poa pratensis* appeared prior to Müntzing's (26) proposal of the occurrence of apomixis in bluegrass. Nishimura (32) discussed the floral primordia, the mature sac, and the young embryonic stages. The critical stages of the reduction divisions and the haploid macrospores were not considered.

Anderson (7) did not report observing the collapse of the macrospores in *Poa pratensis* and *P. compressa* L. Figure B of her plate 2 might suggest that the binucleate embryo sac was derived from a somatic cell lying adjacent to the macrospores. Multiple sacs and polyembryony, features common in apomictic biotypes of *P. pratensis*, suggest that she was dealing with an apomictic form.

MÜNTZING (26), on the basis of progeny tests and chromosome counts on the parent and its matroclinic offspring, concluded that apomixis occurred in *Poa* (*P. palustris* L.). He postulated (22) that variant plants arising in a diploid-parthenogenetic *Poa* species would be haploids.

ARMSTRONG (8) concluded that fer-



FIGS. 1–8.—Fig. 1, plant 73–19; reproduces primarily by somatic apospory. Figs. 2–7, plants 71–10, 72–7, 73–14, 72–18, 72–23, and 72–6, respectively; reproduce primarily by sexual means. Fig. 8, plants 73–1, 9 and 10; plant on right is parental type.

species. In 1935, KIELLANDER (21) reported the direct development of the E.M.C. into the embryo sac (diploid-parthenogenesis) in *Poa serotina* Ehr. tilization takes place in *P. pratensis* because he observed paired chromosomes at the heterotypic metaphase of reduction division in the E.M.C., and because

of good pollen germination. He did not find "any regularity as to the position of the functional megaspore in the row, although it was more frequently the inner one farthest from the micropyle" that developed into the embryo sac. He advanced the hypothesis of random assortment of univalents in the homoeotypic division in *P. pratensis* and also suggested that "only pollen grains of the normal chromosome complement function." In this way an aneuploid number might be maintained in a sexually reproducing strain. The "variation in the position of the functioning megaspore in the row of four . . . . provided a mechanism for the elimination of megaspores with an abnormal chromosome complement and for the choice of a megaspore containing the normal chromosome complement."

ÅKERBERG (1) figured an aposporous embryo sac located laterally in the nucellus to the collapsed macrospores of the ovule primordium of *P. pratensis*. During the later stages of development only remnants of the E.M.C. or its meiotic products were found.

BROWN (11) suggested that "the extent of occurrence of aneuploid forms in different parts of the world may in some degree account for the diversity of opinion concerning the extent of apomixis within the species" (*P. pratensis*). In 1941, he concluded that "the varying units always tend to group themselves into one of two (morphological) complexes," and that "all its peculiarities point to a probable hybrid origin." Two progenies ($2n = 42$) had 18.2 and 16.7% aberrants. Two other progenies ($2n = 56$) produced 1.5 and 2.97% off-type plants. From these observations he concluded that "although the evidence is not conclusive it does indicate that apomixis tends to increase as the chromosome number increases."

MÜNTZING (27) observed that the embryo sac of *P. alpina* develops directly from the macrospore mother cell.

ENGELBERT (14) concluded from a study of the development of twin-embryoed seeds in *P. arctica* that the matroclinic appearance of most plants of a given progeny was due to apospory, whereas the aberrants were the result of "sexual (*n*) embryo" development and occasional cases of fertilization.

TINNEY (40) followed the developmental embryology of apomictic biotypes of Kentucky bluegrass from the macrospore mother cell through to the young ovule. He reported the frequent completion of reduction division, after which the haploid macrospores collapsed. However, collapse sometimes occurred before the division was completed. Simultaneously, a somatic cell (*s*) assumed the function of the embryo sac and developed a functional embryo(s). KIELLANDER (23) and ÅKERBERG (5) reported similar observations from Swedish biotypes of *P. pratensis*.

KIELLANDER (24) reported that a family ($2n = \pm 72$) of *P. pratensis* was morphologically constant throughout three generations. Differences between members of sets of twins were observed. "Two aberrant forms, a twin with $2n = 40$, and a triplet with $2n = 18$ are described." The eighteen-chromosome plant, after open pollination, produced plants with sixteen to nineteen chromosomes. This plant and its progeny resembled *P. trivialis* L.

ÅKERBERG (6) studied the development of the embryo in emasculated florets of apomictic biotypes of *P. pratensis*. Those florets subsequently pollinated developed endosperm, whereas only proembryos and polar bodies occurred in the

unpollinated. He concluded that pollination is necessary for endosperm and functional seed development.

HÅKANSSON (18) discussed the embryology and fertilization of sexual forms of *P. alpina* from Switzerland and apomictic types from Scandinavia. Any of the haploid macrospores of the sexual plants ($2n = 22–25, 31$) was capable of developing into an embryo sac, with some plants producing more than one. The asexual forms showed no evidence of meiosis, in contrast to the sexual forms. The diploid egg cell produced formed the embryo. One embryo was apparently triploid, indicating fertilization—at least in some apomicts. Endosperm formation occurred "only after fertilization of the polar nuclei had been effected (pseudogamy." The embryo, in relation to endosperm development, was observed to start development earlier in apomictic than in sexual forms.

EXPERIMENTAL RESULTS.—

EMBRYO-SAC DEVELOPMENT.—The observations reported here were made primarily on plants that showed marked morphological variations among the members of individual progenies. The conclusions are based on an examination of an average of more than fifty fertile florets of each plant.

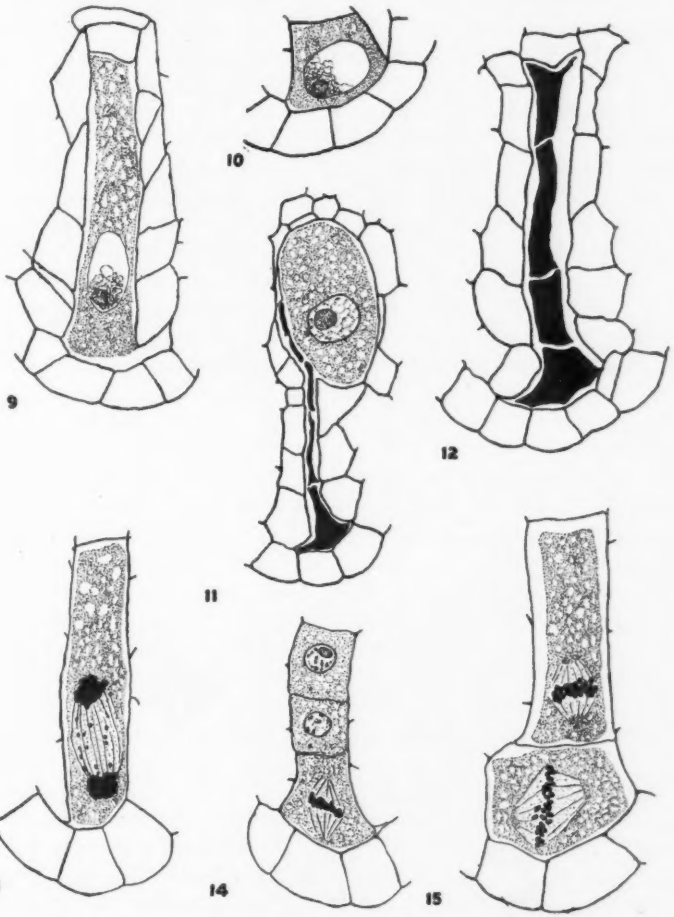
Although the presence of pollen tubes entering the embryo sac has not been observed, the nature of the development of the embryo sac in florets of plants yielding segregating populations makes it probable that fertilization does take place. A few hours after pollination, pollen tubes were frequently observed in the stylar tissue and in the ovarian cavity adjacent to the micropyle.

The macrospore mother cell, located medially under one hypodermal layer of cells of the ovarian primordium, is described by TINNEY as invariably single and elongated (fig. 9). Although this is usual, it is not uncommon to find macrospore mother cells that are only slightly elongated (fig. 10). In a few cases two sporogenous cells have been found lying adjacent to each other. The development of polyembryonic seeds will not be discussed further, other than to indicate the frequency of multiple embryo sacs in the material studied.

The stages leading up to and through the meiotic divisions are usually somewhat irregular. In some instances dicentric bridges and fragments or chromosomes not included in the figure were observed. At the second division it is not uncommon to find the outer of the two daughter cells with an irregular mass of chromatic material irregularly placed in the cell. The innermost daughter cell usually completes its division, forming haploid macrospores. Another alternative is for both nuclei to divide, with their accompanying cell divisions, and to form a lineal tetrad of haploid macrospores. It is during the foregoing stages that collapse occurs, and a uninucleate nucellar cell may take over the functions of the innermost macrospore (figs. 11, 12). Under these conditions development is from a somatic or diploid cell (somatic apospory). When the macrospores collapse and a somatic cell assumes the function of the embryo sac, development proceeds in the manner described by other workers.

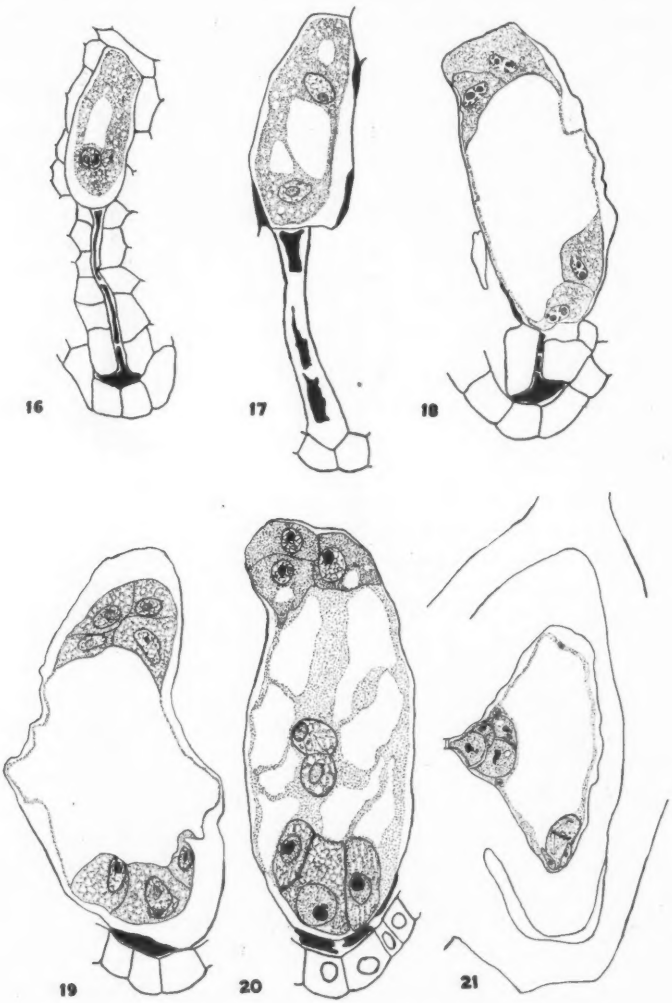
The macrospores of sexual plants sometimes develop according to the "normal type" of embryology. In a fair proportion of cases, the reduction divisions appear quite regular and four haploid macrospores are formed. Figure 13 is illustrative of many of the irregularities observed at anaphase I, and figure 14 shows an excluded body in each of the

two innermost macrospores, whereas in the outermost or micropylar cell most chromosomes are at or near the plate. Although few figures were found wherein the chromosomes in the macrospores could be readily determined, a number of early anaphase figures possessed approximately twenty-eight chromosomes (fig. 15). Generally, however, the tetrad is lineal, the figure shown being an exception. Following the formation of the four haploid macrospores, the three outermost ones collapse and eventually disappear, and the innermost one enlarges



FIGS. 9–15.—Camera-lucida drawings of developmental embryology of *Poa pratensis:* Fig. 9, typical elongate macrospore mother cell; plant 70–11. Fig. 10, typical short macrospore mother cell; plant 72–11. Fig. 11, four collapsed macrospores and enlarged somatic cell of somatic aposporous plant that assumes function of innermost macrospore of sexual plants; plant 71–12. Fig. 12, four collapsed macrospores of sterile floret; plant 71–12. Fig. 13, anaphase I of macrospore mother cell; plant 71–12. Fig. 14, triad with delayed division in the outermost cell of dyad; excluded chromosome present in each innermost cell; plant 72–18. Fig. 15, metaphase II; plant 72–16.

and soon becomes univacuolate (fig. 16). The first gametophytic division occurs when this macrospore is about twice its original diameter (fig. 17). This is followed shortly by two divisions, thus forming a typical eight-nucleate sac (figs. 18, 19). At the division forming the four-nucleate sac, its diameters are about twice those at the preceding division. There is some increase in size by the time



FIGS. 16–21.—Fig. 16, three outermost macrospores collapsed, innermost is univacuolate and uninucleate; plant 72–16. Fig. 17, innermost and functional macrospore is binucleate; plant 72–8. Fig. 18, same, but at four-nucleate stage; plant 72–8. Fig. 19, same, at eight-nucleate stage; plant 70–6. Fig. 20, seven-celled, eight-nucleate stage; plant 70–6. Fig. 21, embryo sac at first zygotic division and after first division of fusion nucleus; plant 72–19.

of the third division, but it is relatively somewhat less than that at the earlier mitoses.

The sac, at the eight-cell stage, has a large central vacuole with four nuclei located in each end of the cell. A protoplasmic belt then forms medially. As the polar nuclei converge toward the center of the sac, bounded on either side by several vacuoles, the synergids become somewhat elongate or pear-shaped, and eventually their cytoplasm may become somewhat fibrillar in appearance (fig. 20). Concurrently the antipodals enlarge and assume a densely staining property and the egg cell becomes somewhat rounded. These three units of the eight-nucleate, seven-celled sac will be discussed separately, although their developmental stages are simultaneous.

The cytoplasmic belt extending from the chalazal to the micropylar end of the sac forms as the antipodals become somewhat rounded in appearance. The cell nearest the funiculus may enlarge to form a haustorium-like structure that perhaps acts as a conductor of food from the provascular strand (fig. 21). The antipodals develop a dense, darkly staining cytoplasmic condition. Scattered throughout these cells are several dark-staining "chromatic" bodies. An occasional division figure was observed. The onset of collapse of the antipodals becomes apparent shortly after the first division of the polar-fusion nucleus. Disintegration and digestion are frequently —but not always—complete by the time the endosperm starts to become cellular.

The polar bodies begin to migrate rather slowly toward each other. Just before the first zygotic division they usually lie free toward the micropylar end of the sac. They then move rapidly toward the egg (or zygote) and fuse when that structure has undergone division. In some instances the polar-fusion nucleus has completed its first division at the time of the first zygotic division. Fusion appears to occur very near or adjacent to the dividing zygote, or the proembryo, after which there is apparently a rapid migration toward the antipodals. The first division of the polar-fusion nucleus usually occurs just above the antipodals. One of the daughter nuclei passes to the distal side of the antipodals (fig. 21), where it divides again at about the same time as its proximal mate. This is followed by a series of rapid divisions, resulting in a free-nucleate endosperm. At this stage the embryo has increased to about sixty cells and the antipodals have usually almost disappeared.

The need of fertilization for endosperm development in somatic aposporous reproducing biotypes has been referred to by several workers (**3, 17, 41**, and others). Plant 71–12, which reproduces largely by apomixis (see Progeny tests), is approximately triploid, with ± 40 "bivalents" (see Microsporocytes). At least 378 (*ca.* 375–400) chromosomes were counted in an early anaphase figure of the polar-fusion nucleus.[5] Since polar bodies in somatic aposporous embryo sacs form from diploid nuclei, they would contribute at least 160 chromosomes to the fusion nucleus. Hence they contributed about 320 members to the anaphase complement, which would leave about 58 (or on basis of *ca.* ± 80) chromosomes unaccounted for except by fertilization of the polar body by an approximately reduced male nucleus. Thirty-seven dividing pairs were counted in about one-fifth of the figure of another meta-anaphase of the fusion nucleus in an embryo

[5] The polar-fusion nucleus at anaphase (71–12) was loosened from surrounding ovarian tissue on a newly prepared slide. This was drawn off and smeared in thin balsam on another slide, making an approximate count possible.

sac of 71–3. The calculated complement of this figure essentially equaled that of 71–12. These observations are in good agreement with those of ÅKERBERG (6), who concluded that endosperm of a Swedish apomictic biotype of *P. pratensis* contained ± 195 chromosomes.

No dividing endosperm nuclei of sexual plants were found wherein the number of chromosomes could be determined. It appears, however, that they contain about one-third as many chromosomes as do the nuclei of apomictic plants. This assumption is based on the comparative sizes of metaphase and anaphase chromosomes and those of the figures of the two types of plants.

In the micropylar end of the sac the egg enlarges and assumes a loosely granular to alveolar structure. As the egg enlarges, the cytoplasm of the synergids may become somewhat longitudinally fibrillar in appearance. They usually remain distended until the formation of the proembryo and then disappear.

In several plants an enlarged somatic cell was observed in the nucellus adjacent to the embryo sac and distal to the micropyle. These cells were first noted when the sacs were mature. They appeared as though stimulated and might have assumed the function of the embryo sac had the haploid sac failed. These extraneous cells were always uninucleate, and their protoplasts were partially plasmolyzed. They looked already in an early stage of disintegration.

EMBRYO SAC FAILURE.—The divergence in number of seedlings developed from seed in single panicles may possibly be explained by the embryology of the developing floret of the mother plant. In some florets the macrospores form and all collapse (fig. 12). Collapse occurs in other florets at the dyad stage. The meiotic divisions of these show considerable irregularity. In most cases the daughter nuclei of the dyads or macrospore tetrad appear partially organized when collapse occurs. Examination of the surrounding nucellar tissue usually exhibited one of two conditions: (*a*) no secondary embryo sacs being formed from nucellar cells; or (*b*) a poorly formed uninucleate sac, similar to those found in most apomictic *Poa* plants. Several factors may have led to these uninucleate somatic sacs being nonfunctional. They were initials when the fertile florets of the spikelet that developed caryopses already had formed cellular endosperm. It would appear doubtful whether they would be able to compete successfully for nutrients with an ovule in a state of storing food.

To determine the significance of embryo sac failure, sectioned material of from twelve to forty florets from each plant was examined. The percentage of functional embryo sacs in florets on each slide was determined. Any embryo sac that had formed and showed no evidence of collapse when fixed was considered functional. For purposes other than this phase of the study, more material was sectioned for some plants than for others. The data were analyzed by analysis of variance to determine whether the variation between plants and that between families was significant. In analyzing data for embryo sac failure, progenies were treated as a whole rather than as morphological groups within progenies. This procedure was followed because the variation within groups appeared to be about the same as that within progenies. Bartlett's chi-square test (X^2) for homogeneity was used to determine whether the mean squares within plants of the several families, and that between plants of the several progenies, were homogeneous (36).

The F values given in table 2 are the ratios of the "between-plant" and the weighted average of the "within-plant" mean squares. Significant F values indicate that the values for the several plants vary more than the average variation within plants. In many cases the X^2 values for the test of homogeneity show that variations within plants are not homogeneous. In order to determine whether any two plants differed significantly in their mean values, either the components of error for these two plants or those components which do not differ significantly among themselves would be used to provide an estimate of the within-plant variation.

Significant F values were found for progenies 71, 72, and 73, indicating differences existing among plants of a family. Likewise, a significant difference was found between families in the amount of sterility. Thus, there is usually a difference in the amount of sterility among plants within families and among families.

The variabilities of sterility among plants of family 72 showed highly significant differences. No differences were found among plants of other families. The P value for comparisons between families exceeded the 5% point.

The percentage of functional embryo sacs was compared also to the frequency of aberrants in progenies developed from seed of these same plants. In florets of families 71–73, with 50% or more functional embryo sacs, the percentage of aberrants decreased as the percentage of embryo sacs increased. This is not true for family 70, where no apparent relationship occurred. Conversely, in those florets having 50% or less functional sacs, the percentage of aberrants was erratic. It appears that as the percentage of functional sacs declines there may be a tendency toward sexuality. Although this seems to be true for the progenies developed from the original selections, it is not necessarily the case for all plants. For example, plant 71–10 is a marked exception, wherein 84% of the embryo sacs are functional but an entirely diverse progeny was developed from its seed.

Likewise, the uniformity of progenies from plants with low percentages of functional embryo sacs is probably due to seeds formed by the incidence of somatic apospory. There appears to be a fair but not general relation between the percentages of functional sacs and the number of seeds produced.

Polyembryony.—The percentages of florets of the several plants, and of the plants of families, having multiple embryo sacs are given in table 2. These data are based upon the number of florets possessing functional embryo sacs and not upon the total florets examined. The data are, generally, what might be expected from asexual and sexual plants of this species; that is, plants reproducing asexually tend to produce polyembryonic florets more frequently than do sexually reproducing plants. Plant 71–10 is a marked exception. In the thirty-one florets of this plant having functional embryo sacs, thirteen florets possessed two, and six florets three or more embryo sacs. In most florets, but not all, one embryo sac was well along toward maturity, whereas the other or others were represented by densely cytoplasmic cells in either a uninucleate or a binucleate condition. The supernumerary embryo sacs were therefore primarily somatic aposporous. Sometimes, however, both embryo sacs representing twins were sufficiently well advanced so that one would expect the embryo of the asexual embryo sac to develop as rapidly as that result-

TABLE 2

ANALYSIS OF VARIANCE, CHI-SQUARE TEST FOR HOMOGENEITY, PERCENTAGES OF FUNCTIONAL AND MULTIPLE EMBRYO SACS, OF EMBRYOLOGY OF POA PRATENSIS

Source of variation	Degrees of freedom	Mean square	Sacs (%)	
			Functional	Multiple
Between plants	7	374.0	.....	.....
Within plants 70– 2	8	321.7	54.9	25.0
6	3	988.2	73.8	15.4
8	9	495.7	73.0	16.3
17	4	90.2	66.6	27.3
18	4	201.5	82.6	31.6
19	2	419.4	54.1	23.1
20	2	1408.3	77.8	0.0
22	3	1031.6	63.0	35.7
Weighted average within plants		454.2	69.9¶	6.4
F† =		0.82		
X^2‡ =		8.10		
Between plants	4	1073.8	.....	.....
Within plants 71– 3	4	82.2	93.3	3.6
6	4	80.0	96.1	20.0
8	1	288.0	46.2	0.0
10	4	266.3	83.8	63.3
12	5	143.1	88.6	15.4
Weighted average within plants		151.0	86.0	20.7
F† =		6.65*§		
X^2‡ =		2.7		
Between plants	16	3656.0	.....	.....
Within plants 72– 1	9	25.5	14.9	0.0
5	5	102.6	68.7	18.8
6	3	438.9	24.3	0.0
7	8	118.3	66.1	0.0
8	16	378.4	66.6	0.0
9	5	125.1	10.0	0.0
11	11	399.1	51.2	0.0
12	4	33.8	12.5	0.0
13	7	689.4	47.5	0.0
14	4	897.8	88.8	9.4
16	8	892.3	50.0	0.0
17	5	401.9	75.6	3.9
18	5	425.1	64.6	0.0
19	8	624.6	46.7	0.0
22	5	224.2	75.0	0.0
23	15	804.2	34.9	0.0
24	13	205.0	67.3	0.0
Weighted average within plants		417.9	49.7	0.1
F† =		8.75**§		
X^2‡ =		44.6**§		

¶ Plants 11 and 22 with 88.9 and 61.2% functional embryo sacs included in weighted average.

† F = ratio of between- to within-mean squares.

‡ X^2 = test of homogeneity of within-plant and within-family mean squares.

§ * = significant 5% level; ** significant 1% level.

TABLE 2—*Continued*

Source of variation	Degrees of freedom	Mean square	Sacs (%)	
			Functional	Multiple
Between plants	10	2497.6	.....	.....
Within plants 73– 1	9	499.4	83.4	12.9
2	14	451.6	80.3	2.7
4	3	50.7	93.9	9.7
5	17	473.2	56.6	1.6
8	10	649.0	54.3	7.3
11	8	106.4	84.8	13.2
14	8	886.2	53.2	4.3
15	10	531.4	52.0	16.1
16	13	248.5	72.1	0.0
19	9	570.4	80.9	10.9
21	9	263.4	80.0	31.8
Weighted average within plants		449.9	71.3	6.9
F† =		5.55*§		
X^2‡=		15.2		
Between families	3	11872.7		
Between plants within families	37	2442.8		
F† =		4.86**§		
X^2‡=		9.21*§		

ing from gametic union. It is readily possible that at least some of the aberrants in the diverse progeny produced by this plant were the result of a somatic aposporous embryo sac being fertilized and would consequently give rise to a plant differing morphologically from its maternal parent.

Microsporocytes

Literature review.—

Reference is made here only to papers wherein the meiotic divisions in *Poa* species are discussed. Kattermann (**20**) reported that *Poa caesia* J. E. Smith (*P. glauca* Vahl.) was meiotically irregular and that univalents sometimes divided during the first division and occasionally failed to divide.

Müntzing (**26**) observed irregularities during meiosis in *P. alpina*. He noted univalents at heterotypic metaphase, as well as multivalent groups. At anaphase I, the univalents split and at anaphase II lagging occurred.

Rancken (**35**) found at least two tetravalent groups in pre-metaphase figures of *P. pratensis*. Pairing was in the form of rings, crosses, and rods, indicating terminal as well as interstitial chiasmata. He did not observe the laggards being reorganized into micronuclei and thus concluded that the excluded chromatic material was reabsorbed into the cytoplasm. Neither did he observe hexads or octets, or other sporocyte groups of more than four members. In addition to the lagging univalents and fragments, he recognized several inconspicuous extraneous bodies.

Flovik (**16**) reported the meiotic divisions of *P. alpigena* type *iantha* ($2n = \pm 77$) and *P. alpigena* type *domestica* ($2n = 83$) to be "generally extremely irregular." Well-organized metaphase plates were observed only occasionally, and laggards were numerous. Micronu-

clei were not common at interphase, indicating that most laggards reached the poles before formation of the daughter nuclei. Laggards of the second division did not reach the poles in time to be included in the quartet nuclei. This often resulted in pentads, hexads, etc. Although individual species differ somewhat in detail from the preceding, FLOVIK observed generally irregular meiosis in *P. alpigena* var. *vivipara* (Malmgr.) Scholander ($2n = 42 + 4ff$); *P. glauca* ($2n = 72$); and *P. arctica*, and *P. arctica* var. *vivipara* (Malmgr.) Scholander ($2n = 56$).

MÜNTZING (**27**) found that in meiotic divisions in a haploid ($n = 18$) plant of *P. pratensis*, 76% of metaphase I figures were "perfectly regular, 1 to 3 univalents being present in the other cells." In 100 A_I cells, where the division was 18–18, there were no univalents in ninety-five of the cells. At interphase only three cells had laggards. He also observed a chromatin bridge and a fragment.

BROWN (**12**) concluded from his study of the same species that "pairing is for the most part, by bivalents, although univalents, trivalents, and quadrivalents have been observed in all cells examined." The univalents, although lagging at anaphase I, "always reach the poles in time to be included in one or the other of the polar groups." He also observed that some plants were "heterozygous for an inversion or duplication as indicated by the occasional occurrence of dicentric chromatids. . . . . "

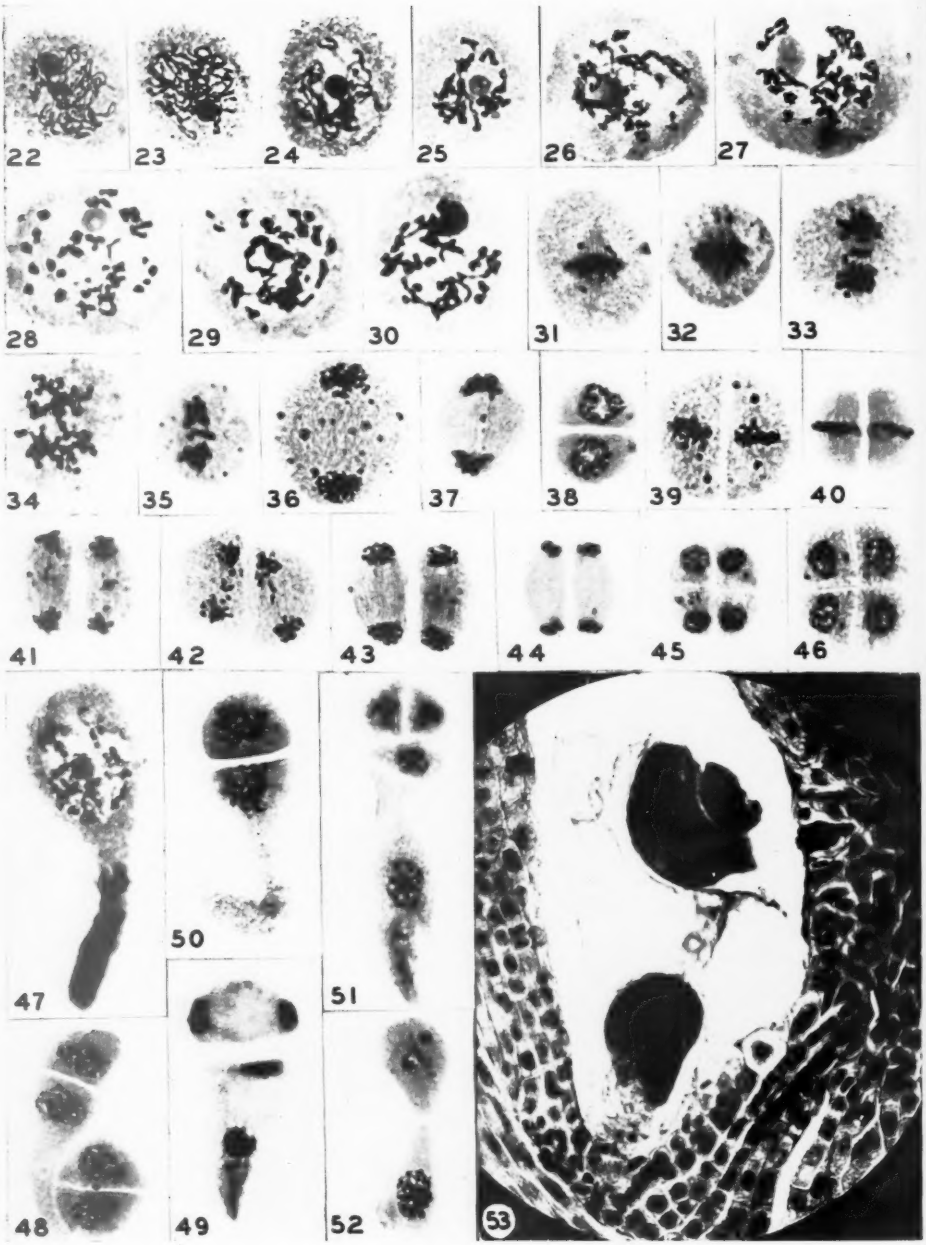
EXPERIMENTAL RESULTS.—

The microsporocytes of twenty-seven individuals were examined. Three plants were first taken at random. One hundred observations were made on these plants at each of the M_I, A_I, T_I,[6] dyad, and quartet stages of development. Twenty-five observations gave sufficient accuracy for comparative purposes. In the section dealing with embryology it was indicated that the plants of segregating populations examined had about twenty-eight bivalents. Microsporocytes of two plants reproducing apomictically had approximately forty bivalents. Since their irregularities are of about the same frequency as the $2n = \pm 56$ chromosome plants, they are not considered separately.

The sporocytes usually contained, in addition to frequent laggards, globular inclusions that took varying amounts of aceto-carmine stain. Representative illustrations are given in figures 22–52. Generally, there was sufficient difference between the staining properties of chromatin and that of the globules to make separation readily possible; other microsporocytes could be studied only with difficulty. The globules may be evident at any stage from prophase through the quartets. They are, however, most conspicuous in anaphase and telophase figures. No plant was entirely free from globules at all stages. The ranges in percentage of cells with globules were at M_I 4–80%, at A_I 4–88%, at T_I 8–80%, at dyad 0–64%, and at quartet 0–44%. Although RANCKEN's (**35**) reference to foreign bodies in *P. pratensis* microsporocytes is indefinite, it is readily possible that the structures he referred to were similar to those figured here.

There were approximately twenty-eight bivalents in most of the material studied. Incident to this number there is also a marked tendency for individual members of the complement to bunch together, making analysis difficult in most figures. It was concluded, therefore, that a determination of the frequency of lag-

[6] The symbols M_I, A_I, and T_I are used for metaphase I, anaphase I, and telophase I, respectively.



[Legends for figures 22–53 on facing page]

gards was sufficient for this study. The data from such an examination are given in table 3. These, together with representative forms shown in figures 22–52, are illustrative of meiosis in the material examined.

During the prophase stages the chromatids appear to be normally paired (figs. 22–24). At diakinesis many of the figures exhibited disturbances. Some of the chromosomes shown at 5:00–6:00 o'clock in figure 28 are unpaired. Chromatin strands were evident in figures 27–29. Whether such figures are characteristic of the plants or due to an early stage of necrosis of the sporocytes was not determined. Figure 27 contains also an association of a ring of four chromosomes. Other multivalent groups of three and four chromosomes may be seen in several of the figures.

The data for lagging univalents at M_I, A_I, T_I, dyad, and quartet stages were analyzed similarly to those for embryo sac failure. The results are summarized in table 3. The mean number of laggards at each of the above-mentioned stages of development are also included. In some cases the number of laggards at M_I is somewhat lower than that at A_I. It is likely that a part of this difference may be due to some chromosomes being considered on the plate when actually they were not. There is also the possibility that all chromosomes were not included in the spindle, or that chiasmata failed to terminalize, thus leaving non-congressing members in the plate region. The ranges of microsporocytes without laggards were at M_I 0–32%, at A_I 0–20%, at T_I 0–56%, at dyad 12–100%, and at quartet 16–100%. The data show that differences are not entirely consistent for all stages. Significant differences were found in the meiotic behavior between plants within several families for all stages except A_I of families 70 and 71, and the dyad and quartet stages of the latter progeny. No differences were found between the mean values of the several families for any of the characters.

Significant differences among plants for the within-plant mean squares were determined for all stages of development (excepting M_I) of progeny 70, M_I, T_I, and dyad of progeny 71. With certain exceptions, values are usually highly significant, indicating heterogeneity; thus certain plants are more variable than others. Highly significant X^2 values for the average mean squares within plants between progenies were found, whereas the X^2 values between plants within families were not significant.

In addition to these meiotic irregularities, bridges frequently—but not always—accompanied by an acentric fragment were observed. Eighteen such bridges were seen. Three occurred in microsporocytes of plants 70–8, 71–3, and 73–2. Two occurred in sporocytes of 71–8, 72–9, and 72–14. There was one each in 70–

FIGS. 22–53.—Sporocytes of *Poa pratensis:* Fig. 22, prophase; plant 70–22. Fig. 23, prophase; plant 70–22. Fig. 24, prophase; 73–11. Fig. 25, diakinesis; 70–22. Fig. 26, diakinesis; 73–14. Fig. 27, diakinesis; 73–14. Fig. 28, diakinesis; 73–14. Fig. 29, diakinesis; 73–14. Fig. 30, diakinesis; 73–14. Fig. 31, metaphase I; 73–21. Fig. 32, metaphase I; 72–6. Fig. 33, anaphase I; 70–22. Fig. 34, anaphase I; 72–16. Fig. 35, anaphase I; 72–6. Fig. 36, telophase I showing globules; 70–22. Fig. 37, telophase I; 73–21. Fig. 38, dyad; 73–21. Fig. 39, metaphase II; 70–22. Fig. 40, metaphase II; 73–21. Fig. 41, late anaphase II; 70–22. Fig. 42, late anaphase II; 70–22. Fig. 43, telophase II; 70–22. Fig. 44, telophase II; 72–6. Fig. 45, quartet; 70–22. Fig. 46, quartet; 73–11. Fig. 47, anomalous sporocyte at diakinesis; 72–16. Fig. 48, anomalous and normal dyads; 73–21. Fig. 49, anomalous sporocyte at telophase II; 73–21. Fig. 50, anomalous sporocyte at early anaphase II; 70–22. Fig. 51, anomalous quartet with secondary division figure in lower projection; 70–22. Fig. 52, anomalous sporocyte with bivalent, univalent, and nucleolus in upper cell; 7[illegible]–1. Fig. 53, anomalous embryo sac with misplaced antipodal; 70–22.

TABLE 3

AVERAGE LAGGARDS PER SPOROCYTE, ANALYSIS OF VARIANCE, AND CHI-SQUARE TEST FOR HOMOGENEITY IN POA PRATENSIS

Source of variation	Metaphase I		Anaphase I		Telophase I		Dyad		Quartet	
	¶I	Ms	I	Ms	I	Ms	I	Ms	I	Ms
	Between and within plants, for the several families									
Between plants	.....	31.53	.....	19.62	.....	50.8	.....	14.3	.....	5.3
Within plants:										
70– 8	4.7	9.04	3.9	13.10	2.5	3.18	2.4	4.69	1.2	2.33
11	2.6	11.75	6.4	8.08	5.1	14.49	1.6	3.25	1.6	4.75
13	5.5	6.25	4.2	3.04	2.8	4.48	1.1	1.69	0.9	1.33
17	6.2	6.63	4.1	3.17	2.8	2.90	1.2	1.39	1.3	2.46
18	4.5	5.92	4.8	7.75	5.3	10.63	3.3	5.14	1.3	2.63
20	4.8	7.04	5.2	6.87	3.0	4.63	2.1	4.61	2.2	4.47
22	4.1	6.71	4.2	4.33	1.3	2.64	2.0	3.08	0.8	1.61
Weighted averages within plants	4.6	7.62	4.7	6.63	3.3	6.06	1.9	3.41	1.3	2.80
F¶=		4.14**‡		2.96		3.38**		4.20**		1.90
X²†(6 df)=		4.5		20.6**		31.9**		17.0**		16.0*
Between plants	.....	22.41	.....	7.80	.....	18.17	.....	3.70	.....	2.73
Within plants:										
71– 3	4.6	3.08	3.0	3.25	1.8	2.73	1.5	2.18	1.0	1.08
8	2.8	3.12	2.4	1.74	0.8	0.94	0.6	0.92	0.2	0.27
10	2.4	3.54	2.7	6.29	2.0	2.46	0.9	1.58	0.8	2.14
12	3.2	3.48	3.7	5.31	2.8	2.14	0.9	1.16	0.6	0.75
Weighted averages within plants	3.3	3.31	2.9	4.15	1.9	2.06	1.0	1.46	0.7	1.06
F¶=		6.77**		1.88		8.80**		2.54		2.57
X²†(3 df)=		0.60		10.6*		7.2		5.0		12.5**
Between plants	.....	27.31	.....	43.81	.....	22.59	.....	18.76	.....	5.1
Within plants:										
72– 1	4.2	2.25	3.1	4.44	2.0	5.96	1.4	2.25	1.3	2.06
5	5.3	6.46	6.2	7.75	4.1	4.26	2.6	3.50	.8	1.08
7	2.0	2.83	2.4	3.50	2.1	5.07	1.6	4.92	1.0	2.71
9	5.2	20.58§	4.2	4.31	2.4	3.75	1.2	1.39	1.2	2.39
13	3.0	2.29	3.4	2.76	3.0	0.83	0.0	.....	0.04	.....
14	3.9	3.11	4.1	4.74	1.4	1.99	1.5	1.76	0.4	0.42
16	3.0	6.63	6.3	19.96§	4.1	20.56§	3.8	8.23§	1.2	1.17
17	3.9	4.49	3.8	6.67	2.3	3.54	2.2	2.81	1.2	0.88
23	4.0	3.63	5.0	4.88	2.7	2.38	1.8	1.81	0.2	0.36
Weighted averages within plants	3.8	5.81	4.3	6.56	2.7	5.37	1.8	3.33	0.8	1.23
F¶=		4.70**		6.68**		4.20**		5.63**		4.15**
X²†(8 df)=		57.9** (15.3)		38.5** (8.8)		77.8** (27.5)		31.6** (14.0**)		41.8** (41.4**)

¶ F = ratio of the between- to the within-mean squares.

† X^2 = test of homogeneity of the within-plant and within-family mean squares.

‡* = significant at 5% level; ** significant at 1% level. § Removed from calculation for X^2 value given in parentheses.

TABLE 3—*Continued*

Source of variation	Metaphase I		Anaphase I		Telophase I		Dyad		Quartet	
	I	Ms	I	Ms	I	Ms	I	Ms	I	Ms
	Between and within plants, for the several families									
Between plants	.....	98.53	.....	31.33	.....	32.58	.....	6.50	.....	16.50
Within plants:										
73– 1	6.7	10.04	4.0	1.33§	2.2	2.97	1.4	1.83	2.4	2.91
2	7.0	5.04	5.2	5.23	3.1	5.03	1.2	1.69	0.12	1.61
5	3.8	2.69	6.8	6.69	4.8	9.03§	2.4	4.83§	2.6	4.24§
11	3.2	3.48	3.2	3.17	2.2	1.73	0.9	1.28	1.0	1.54
14	2.0	4.13	4.9	8.78§	4.0	5.21	0.8	0.83	1.0	1.46
15	6.4	11.42§	4.2	3.98	1.6	2.42	1.3	1.81	1.0	2.00
21	3.8	3.17	4.7	3.46	2.4	1.68	1.3	2.46	0.4	0.67
Weighted averages *within* plants	4.7	5.71	4.7	4.66	2.9	4.01	1.3	2.11	1.4	2.06
F¶ =		17.26**		6.72**		8.13**		3.08**		8.01**
X^2†(6 df) =		24.3**		23.3**		28.7**		51.8**		22.9**
		(14.8*)		(4.4)		(14.6*)		(7.6)		(12.4)
	Average within plants for several families									
		Ms		Ms		Ms		Ms		Ms
X^2†(3 df) =		18.8**		12.0**		34.9**		28.6**		38.6**
	Between families and between plants within families									
	df	Ms	df	Ms	df	Ms	df	Ms	df	Ms
Between families	3	69.26	3	84.5	3	43.90	3	34.6	3	20.10
Between plants within families:										
70	6	31.53	6	19.50	6	50.83	6	14.30	6	5.32
71	3	22.40	3	7.80	3	18.17	3	3.70	3	2.73
72	8	27.31	8	43.81	8	22.59	7	18.76	8	5.10
73	6	98.53	6	31.33	6	32.58	6	6.50	6	16.52
Weighted averages within plants		46.35		29.52		31.99		12.14		7.83
F¶ =		1.50		2.86		1.37		2.85		2.57
X^2† =		3.9		2.69		1.46		3.05		4.13

17, 71–10, and 73–21. Thus at least nine of the twenty-seven plants studied may have been heterozygous for an inversion or duplication.

These observations upon quartets differ from those of BROWN (12). He reported seeing no micronuclei in quartets, whereas in the data reported here only one plant was entirely free from this irregularity. In only three of the plants examined were 75% or more of the sporocytes without micronuclei.

Multiple sporocytes were observed in plants 72–5, 72–9, and 73–11. These included two triads, one pentad, five hexads, and one heptad. The numerous meiotic irregularities would lead one to assume that multiple sporocytes would be observed more frequently.

There does not appear to be any direct relationship between the frequency of laggards during meiosis in microsporocytes and the breeding behavior. Neither does this frequency of laggards appear to be directly related to embryo sac collapse.

ANOMALOUS STRUCTURES

Certain anomalies have been observed of which only passing mention will be made.

A microsporocyte approximately at diakinesis is shown in figure 47. A cytoplasmic "tube," most of which is out of focus, is also evident. Two sporocytes at M_I united by a cytoplasmic tube were also observed. That some of such anomalous sporocytes complete their division is evidenced by the dyad shown in figure 48, early A_{II} in figure 50, telophase$_{II}$ in figure 49, and the quartet stage in figure 51. The latter photograph shows a secondary division figure involving approximately four chromosomes in the lower left projection. Likewise, the "sporocytes" shown in figure 52 are peculiar, since the lower cell was at one of the prophase stages. The upper cell shows a nucleolus, a bivalent, and a univalent. Such a configuration may represent a variation of the abnormalities, described earlier in this paragraph. If so, three laggards were apparently left in the protuberance; hence, the figure of a bivalent and univalent, neither of which is in direct association with the nucleolus. The origin of these structures is not clear. There is no evidence that they arise in any particular portions of the thecae. Conversely, in plant 73–21, where such structures were relatively common, they were observed twice in medial portions of unbroken sporogenous tissue but not in terminal or end positions.

Figure 53 shows a longitudinal section of an ovule. One of the antipodals of the embryo sac is located in the micropylar end rather than laterally toward the chalaza of the developing ovule. A normal cytoplasmic membrane was evident surrounding the displaced antipodal. The floret shown was probably apomictic, since it may be observed that a proembryo of approximately sixteen to twenty cells had formed prior to the first division of the fusion nucleus—which cannot be seen in the plane photographed. The anomaly figured here is essentially similar to that recently reported by HÅKANSSON (18).

Discussion

The data that have been presented pertain primarily to processes consequential to apomixis in *Poa pratensis*. They do not explain causal phenomena. Throughout the data the cytogenetic instability of closely related plants has been evident. There have been marked and inconsistent variations among the different plants in each of the phases of the life cycle studied.

Classification of plants on a morphological basis was found to be unrelated to the development of seedlings in progeny tests. Although plants were considered to be morphologically similar, differences occurred in both number and vigor of seedlings. The data suggest that these characters are independent and that it does not necessarily follow that vigorous plants will produce numerous or vigorous seedlings.

There was, generally, no direct relationship between plant morphology and breeding behavior. Individual plants, either weak or strong, might produce a morphologically constant or a segregating progeny. Hence, it is not possible to predict, or valid to assume, that morphologically similar plants will behave in a concomitant manner as expressed by breeding behavior. Neither does there appear to be a direct relationship between the number of seedlings produced, or their vigor, and the uniformity of a random population of mature plants grown from those seedlings.

Analysis of data concerning the meiotic divisions in microsporocytes aligns itself with the foregoing statements. For the most part, significant differences in irregularity among plants of the same progeny were determined. Similar differences also were ascertained among related progenies.

The incidence of the collapse of developing macrosporocytes is suggestive of not being related to plant type or other characters. A complicating factor enters, however, with the introduction of an asexual type of reproduction. The limited data available at a high incidence of embryo sac collapse are inconclusive, but they are suggestive, none the less, that no direct relationship exists between this factor and somatic apospory in *Poa*. With the introduction or assumption of apomixis, the picture is largely hidden. However, as progenies become more uniform there is also an increase in the frequency of functional embryo sacs.

Several workers have suggested that *P. pratensis* is of hybrid origin (**12, 23,** and others). Present studies lend strong support to the assumption. The meiotic irregularities observed in microsporocytes are essentially similar to those reported for known grass hybrids (**33, 34, 29, 19, 25,** and others). Concomitantly, the meiotic irregularities in the developing macrospores are sufficiently great to lead to almost complete sterility in some plants. This sterility and the meiotic irregularities in the sporocytes suggest that somewhat remotely related genomes may have been involved in the synthesis of the species. As a result of his study of a haploid twin *P. pratensis* plant, KIELLANDER (**24**) suggested that *P. trivialis* may have been involved as one of the species in the cross. Among twins isolated by this writer is one $n = 14$ (unpublished data). This plant bore no resemblance to *P. trivialis*.

It has been shown by several workers that progenies developed from field collections are predominantly constant morphologically. Occasionally such a collection produces a heteromorphic progeny when grown as spaced plants. The aberrants occasionally reproduce apomictically, but predominantly they develop heterogeneous progenies, many plants of which are weak and perhaps would soon disappear from a mass-seeded population. Some, however, are sufficiently vigorous to appear able to compete successfully with their usually more vigorous, apomictic types. The possibility of reduced and unreduced female gametes being fertilized also exists (**18**). These unreduced gametes, which have been developed from a somatic cell, may

be either eu- or aneuploid. When they are fertilized, biotypes of chromosome numbers different from those of the parents arise. Whether or not *Poa* pollen of aneuploid chromosome number is functional is not definitely known. If so, further aneuploidy may be promulgated. It is only when complementary sets of chromosomes are joined that constancy of cytological behavior might be expected. Judging from the data presented, this is only occasional, and hence there occurs in the species a range of chromosome numbers varying from eighteen to well over 100 (**9**, **38**, and others).

The fact that there are frequent meiotic disturbances of *Poa* resulting in reduced fertility in apomictic types is overcome by the asexual form of reproduction assumed by the species. It is only when factors for apomixis (the development of somatic "gametophytes") are absent that a reasonably accurate measure of the genetic make-up of *P. pratensis* as a probable allopolyploid species is possible.

There is good agreement between the data presented and that of MÜNTZING and MÜNTZING (**28**). They were able to synthesize a "new constant biotype of the basic chromosome number of the genus" by crossing certain strains of *Potentilla opaca* L. onto segregates of *P. argentia* L. Principal analogies between plants and progenies from controlled crosses of sexual, partially apomictic and apomictic, biotypes of *Potentilla* and those from open-pollinated *Poa* plants may be summarized as follows: (*a*) New constant lines are established from aberrants with approximately the diploid and triploid chromosome number; both types are apomictic, one arising by an internal genetic change (**28**) and the other by fertilization of an aposporous egg cell. (*b*) The poor seed set in some *Poa* plants is essentially similar to that known for known interspecific crosses in *Potentilla*. (*c*) Irregularity in meiosis is due to hybridity, whereas constancy in morphological type is due to apomixis. (*d*) The occasional occurrence of sexually reproducing florets in apomictic plants, and its converse, result in the diversity of naturally occurring populations of these species.

Summary

1. Germination studies carried on in soil in pans, and in a seed germinator, indicated wide average differences among plants of *Poa pratensis* in the number of seeds set and also in the viability of such seeds. Although germination percentages of a few plants were less than 10%, most plants produced seed of good viability. The poor seed set of some plants was due to frequent collapse of the developing macrosporocyte during or just after meiosis. Significant differences in the amount of such sterility was determined for between-progeny comparisons. Plants within a given progeny were significantly different among members in one progeny, that is, 72.

2. Forty-four progenies from as many plants of four related parental families were grown. The frequencies of aberrant plants among the second-generation individuals varied from zero to 100%. There did not appear to be any direct relation between the morphological characteristics of parent plants and the breeding behavior of their progenies. This also appears true for other characters, such as seed set, germination, etc.

3. Globular inclusions occurred in the developing microsporocytes of all plants. These were particularly prominent during anaphase and telophase stages, although they were evident in all stages from prophase through the quartets. Highly significant differences generally

were found in microsporocytes in the frequency of laggards among plants, but not always.

4. Certain anomalies among developing ovules and microsporocytes were observed and figured. Their origin was not determined.

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GROWTH AND DEVELOPMENT IN RANGE GRASSES. V. PHOTOPERIODIC RESPONSES OF CLONAL DIVISIONS OF THREE LATITUDINAL STRAINS OF SIDE-OATS GRAMA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 565

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Introduction

Earlier work (6) has shown that twelve geographic strains of side-oats grama (*Bouteloua curtipendula* [Michx.] Torr.) are decidedly differentiated in their photoperiodic responses, as judged by their behavior over a 2-year period on Chicago natural daylength and on constant daily photoperiods of 9, 13, 16, and 20 hours. Three strains from southern Texas and Arizona apparently consist largely of intermediate- or short-day plants, while a strain from North Dakota is probably made up chiefly of long-day plants. The other eight strains, from Nebraska, Kansas, Oklahoma, and New Mexico, seemed to include numerous long-day individuals, although the length of the critical photoperiod for the "late" plants decreased with decrease in latitude of origin. The Oklahoma and New Mexico strains showed considerable diversity in response within each strain on each treatment, and may include both intermediate- and long-day plants. These studies and conclusions were based on populations of twenty to fifty individuals of each strain on each of the five treatments.

It seemed desirable to determine somewhat more precisely the degree of diversity in photoperiodic response within some of these strains, and to narrow the range of treatments so that the results might better indicate the length of criti-

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cal photoperiods and the practical importance of photoperiod in the seasonal and latitudinal adjustment of these strains. Accordingly, clonal divisions of twelve selected individuals from each of three strains were grown during 1944 on Chicago natural daylength and on constant photoperiods of 13, 14, and 15 (or more) hours. Thus, genetically identical populations were grown on each treatment.

The necessary background of related studies by other investigators, the reasons for this general type of investigation and choice of species, and the values which may be derived, have been discussed in previous papers (**5, 6**) and will not be repeated here.

Material, methods, and environmental conditions

Strains 1, 5, and 12 of the previous report (**6**) were selected for investigation in 1944, since they represent the extremes and approximate means of latitudinal origin (approximately $29\frac{1}{2}$, $35\frac{1}{2}$, and $46\frac{1}{2}$ degrees N) and range of response of the twelve strains grown in 1942–43. Strain 1 originated at San Antonio, Texas; strain 5 at El Reno, Oklahoma; and strain 12 at Cannonball, North Dakota. On April 16, 1944, twelve individuals from each strain were picked from populations of 2-year-old plants which had been grown chiefly on Chicago natural photoperiod until late December, 1943. These individuals were chosen so as to sample the variation previously shown by these populations in vegetative and reproductive habits, including early and late flowering (or nonflowering), on Chicago natural photoperiod.

In the autumn of 1943, plants of strains 5 and 12 had become dormant in a warm greenhouse, dying back to within 2–3 cm. of the ground, strain 1 to a lesser degree, apparently in response to decreasing light intensity and photoperiod (**6**). In late December these plants were given supplementary illumination to provide a 16-hour photoperiod. They grew slowly in January, and by mid-February new foliage averaged 15–20 cm. tall. On March 29 these plants were again placed on natural photoperiod, when they ranged from 30 cm. (strain 1) to 20 cm. (strain 12) in height. No flowering and little culm elongation had occurred by April 16, when the selected plants were clipped back to an average foliage height of 7.5 cm. at the time of transplanting and division.

Each selected individual was divided into four equal pieces, the old roots being pruned to a length of 2.5–5 cm. The divisions were planted singly in 8-inch unglazed clay pots filled with a silt-loam soil. This volume of soil was apparently adequate to allow vigorous development of each division, so that it is unlikely that either the vigor of flowering or the length of the flowering season was limited by this factor. It is probable that the effects of clipping and root pruning may have somewhat delayed subsequent floral initiation, since one of the reserve unclipped individuals of strain 5 on natural photoperiod exserted an inflorescence on June 1. This may be compared with the appearance of the first inflorescence on July 22 in the clipped plants on natural photoperiod in the experiment, while all other clones of this strain and series flowered only after August 2. In the 13-hour series, however, all exserted their first inflorescences between June 26 and August 5. This suggests that possibly some of the experimental plants of strain 5 on natural photoperiod might have flowered much sooner if they had not been relatively defoliated during exposure to effective photoperiods ranging

from 14 to 15 hours in late April and early May—possibly more favorable to them for floral initiation than the longer ones of June. In previous experiments unclipped plants of strain 12 on natural photoperiod exserted inflorescences as early as May 15, in contrast with June 26 for the clipped plants of the experiment. No plants of strain 1 at Chicago, however, have flowered in spring or summer on natural photoperiod.

The meristems of a large number of unclipped plants comparable with those selected for clonal division were examined with a dissecting binocular microscope on April 16. No inflorescence primordia were found in any of the strains. It is thus probable that inflorescences appearing in 1944 in both clonal divisions and reserve plants were initiated after April 16 while on the photoperiods on which they were placed on that date, and not during some preceding period. It had been shown previously (**5**) for a different strain that clipping apparently removes the possibility of much aftereffect of previous photoperiods. This would seem to be even more the case when floral primordia, which might escape clipping, are not already present, since it has been shown repeatedly for certain species (**1, 2**) that the photoperiodic stimulus to flower is exerted through the leaves and then transmitted to the meristems.

The four divisions of each individual of each strain were distributed to four trucks or benches to receive photoperiods of 13, 14, and 15 hours, and Chicago natural daylength (N series). The 13- and 14-hour series, on adjacent movable trucks, received natural daylight for 9 hours between 8:00 and 5:00 P.M. (CDT) but were rolled into ventilated lightproof sheds for the remainder of the 24 hours. The 13-hour series received 4 hours of artificial light from 5:00 to 9:00 P.M., while the 14-hour series was given 5 hours between 5:00 and 10:00 P.M. These treatments thus differed by only one hour of artificial light. The intensity of supplementary light, from 200-watt Mazda lamps mounted in individual reflectors, varied from 100 to 180 footcandles at average foliage height. The 15-hour series on a stationary truck in the same room received supplementary light after sunset, when necessary, to provide a photoperiod of 15 hours or more. Between June 2 and July 19 this series was left on natural daylength only. Thus for a time in late spring and early summer it was on photoperiods ranging between 15 and 16 hours, but after August 1 the effective photoperiod was little more than 15 hours. The N series was in a separate greenhouse room similar in temperature and natural light intensity conditions to that containing the other three series.

Responses on Chicago natural daylength may be usefully compared with behavior under field conditions and with the results obtained in similar series in the two preceding years. Thirteen-hour treatments were also used previously. This photoperiod is near or below the lower limit of the photoperiodic range on which these strains flower under natural conditions. The North Dakota strain is subjected to photoperiods of 14–17 hours or more during most of its natural growing season, while effective natural daylength in Texas and Oklahoma ranges between 14 and 15 hours (possibly $15\frac{1}{2}$ in Oklahoma) for a much shorter time in late spring and early summer. Maximum photoperiods (sunrise to sunset at the summer solstice) for the three strains are approximately 14 hours (Texas), 14 hours and 35 minutes (Oklahoma), and 15 hours and 50 minutes (North Dako-

ta). Thus critical photoperiods of significance in the native environments for either short- or long-day plants of these three strains might be expected to fall very near or within the relatively narrow range of treatments of this experiment, although the status of intermediates would not be completely clarified.

Growth was resumed fairly rapidly after transplanting by most of the clonal divisions. Most of them, with the exception of strain 12 on 13-hour treatment, grew vigorously. A few failed to become established, chiefly in strain 1. Data presented are based on the clones surviving and growing well on at least three of the treatments.

After the emergence of the first inflorescence above the inclosing leaf sheath on June 13, records were kept of the exsertion of inflorescences and their anthesis on each clonal division at intervals of 3–7 days for the ensuing 3 months and at longer intervals thereafter until November 17. On this date flowering had ceased on practically all plants on all treatments. Most of them in strain 12 and many in strain 5 had stopped growing, having become dormant and dying back as in previous years (6), although the plants on 15-hour treatment on this date had a greater proportion of green foliage than those on other photoperiods. Strain 1 had become dormant to a lesser degree, with 60–90% of the foliage still green on all treatments. Some tillers were still growing very slowly. Even the apical meristems of the oldest and longest culms were still alive and somewhat active in the 14- and 15-hour series.

If external conditions control the diminished activity and dormancy of this species in the autumn under greenhouse conditions, it seems apparent that growth—and especially flowering—can proceed vigorously only when natural light intensities are above the levels common in the greenhouses at Chicago in the autumn and winter months, no matter how favorable the temperature and photoperiodic conditions. However, while seasonal light intensities at Chicago are lower than those to which these strains are subjected under natural conditions, the vigor and habits of the plants (figs. 2–10) on most treatments in the summer suggest that the intensities at Chicago in spring and summer fall within the favorable range for this species for both vegetative and reproductive behavior. The reported photoperiodic responses during these seasons, therefore, are probably not unlike those which would be obtained on similar photoperiods in the native environments of the strains, since the other critical environmental values in these experiments simulate those of the places of origin, so far as they might condition the reported responses. The results may thus be interpreted rather generally.

Results

Flowering seasons

As in previous experiments, the beginning and progress of flowering were measured by the exsertion of inflorescences from the surrounding leaf sheaths. While initiation of inflorescences probably occurs 2–4 weeks before exsertion, time and material did not permit the dissection necessary to show it. So far no other criteria have proved satisfactory for recognition of reproductive activity in these strains prior to exsertion of the first inflorescence. Anthesis followed exsertion in 7–21 days, generally after 10–14 days —except in the last few weeks of the experiment, when it was longer delayed.

The extremes and mean number of days after April 16 required by the clonal divisions which flowered to exsert their

first inflorescences are given in table 1. The ascending curves in figure 1 also indicate the diversity shown among the clones within each strain on each treatment in this respect, as well as the final percentage of flowering plants in each population. Figure 1 also summarizes data on the duration of the seasons of inflorescence exsertion by the various strains on different treatments, showing the percentages of plants which were actively exserting inflorescences at various times during the season. Table 2 also shows the extremes and average duration of the periods of active exsertion by individual clones. The relative production of inflorescences in each strain and treatment, based on the average of all individuals in each population, whether flowering or nonflowering, is shown in table 3. The total number of clones, rather than that of the flowering ones in each treatment, was used in the computation, since interest is centered on the vigor of flowering by each population rather than on individual clones.

TABLE 1

NUMBER OF DAYS AFTER CLONAL DIVISION AND CLIPPING ON APRIL 16, 1944, UNTIL FIRST FLOWERING ON DIFFERENT PHOTOPERIODS. AVERAGES AND EXTREMES BASED ON PLANTS WHICH FLOWERED IN TWELVE CLONES OF EACH STRAIN

STRAIN NO.	PHOTOPERIODS (HOURS)			
	13	14	15	N
12 Extremes	91–129	64–97	71–104	71–91
Average	113	77	83	78
5 Extremes	71–115	84–122	108–173	97–143
Average	91	104	132	124
1 Extreme	58–84	143–194	None	157–160
Average	69	179	None	158

TABLE 2

LENGTH OF SEASON (IN DAYS) IN WHICH INFLORESCENCES WERE BEING EXSERTED BY INDIVIDUAL CLONES (NOT DURATION OF EXSERTION BY EACH POPULATION)

STRAIN NO.	PHOTOPERIODS (HOURS)			
	13	14	15	N
12 Extremes	1–42	18–96	38–131	31–65
Average	9	62	76	47
5 Extremes	18–77	1–97	1–65	1–45
Average	46	47	21	22
1 Extremes	89–129	7–58	0	13–23
Average	109	29	0	19

TABLE 3

AVERAGE* NUMBER OF INFLORESCENCES PRODUCED PER PLANT BY POPULATIONS OF IDENTICAL CLONES IN EACH STRAIN ON EACH PHOTOPERIODIC TREATMENT

STRAIN NO.	PHOTOPERIODS (HOURS)			
	13	14	15	N
12	1.8	19	19	16
5	8	8	1.7	2.9
1	31	5	0	16.3

* Based on whole population, not on flowering individuals.

STRAIN 1.—The data of tables 1 and 2 and of figure 1 indicate that the selected plants of this strain from Texas are similar with respect to the criteria shown. Since they are also very similar in appearance, only one clone is illustrated (fig. 2). They are obviously intermediate- or short-day plants, as shown by the absence of inflorescences on 15-hour photoperiod and the delay in exsertion until September 20–23 in the N series, when inflorescences appeared on all clones. In the previous experiments (6) they first appeared on September 28 on natural photoperiod. The early and long-continued flowering in the 13-hour series in all experiments, and the long-delayed

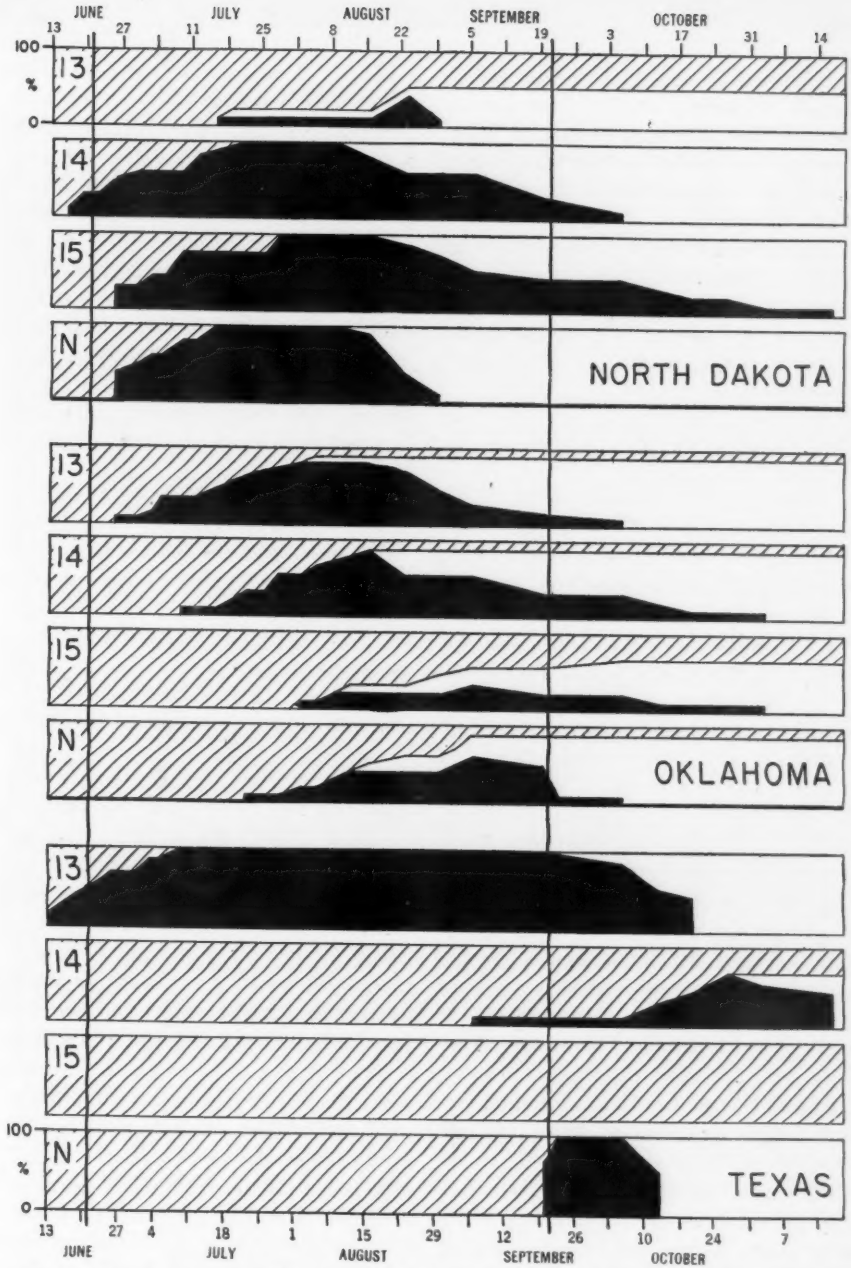


FIG. 1.—Flowering seasons at Chicago of identical clonal populations of each of three strains of side-oats grama, grown after clipping and division on April 16, 1944, on photoperiods of 13, 14, and 15 hours, and natural daylength (N). On any particular date, the value shown by the shaded area is the percentage of plants actively exserting inflorescences; hatched area, percentage which had not flowered; and white area, percentage which had ceased flowering.

and limited flowering on 14-hour photoperiod, strikingly confirm this conclusion. All the data suggest that an upper critical photoperiod for rapid and vigorous flowering lies slightly below 14 hours. Thus the initiation of the first inflorescences to be exserted in the N series must have occurred near the last week in August, when the effective photoperiod at Chicago first falls to this level after the summer solstice (the period between sunrise and sunset on August 31 is 13 hours and 12 minutes). The similar decline to effective photoperiods of approximately 14 hours in southern Texas occurs in early August. During the early part (March, April) of the average growing season at San Antonio, natural photoperiods are also below this level. Information has not been obtained on the time of initiation of growth or of inflorescences by this strain in that area. Seed is ripe by September 15 at San Antonio. Under the warm temperature conditions, inflorescences bearing it might well ripen within 6 weeks after an indicated initiation in late July or early August.

The intermediate- or short-day status of these clones has not been determined, even though exsertion of new inflorescences ceased on October 13 in the N series. These last inflorescences to appear must have been initiated on effective photoperiods of $12\frac{1}{2}$–13 hours or more. The cessation of flowering, however, was probably not due to the decrease of the photoperiod to an unfavorable length for initiation, since previous experiments have shown that individuals in this strain will grow and *some* (but not all) will flower on daily photoperiods of 9 hours in the summer months at Chicago, although much less vigorously than on 13-hour photoperiods. A 9-hour photoperiod is below the level to which they are naturally subjected, even when dormant. Rather, the short flowering season and cessation of flowering in the N series occurred as a consequence of the apparently nearly simultaneous transformation of *all* apical meristems on tillers of any considerable size into inflorescence primordia in response to the decrease of daily photoperiods to a certain length in August and September. Dormant buds and small tillers which might have prolonged the reproductive activity had either not attained a size favorable for initiation or were inhibited in growth as a consequence of the flowering of the older culms.

Inflorescences in the N series thus appeared simultaneously on culms which began to elongate soon after transplanting in April and on the larger of those which developed later during the summer. As a consequence there was a wide gradation in mature culm length and in the number of elongated internodes per flowering culm at anthesis, the longest and oldest ranging from 80 to 90 cm. long with eight to eleven elongated internodes, while those just beginning to elongate at the time of floral initiation attained lengths of 50–60 cm. with five or six elongated internodes. It is obvious that internodal elongation may precede and is neither a sign nor an effect of reproductive activity in this strain. Stems as long as 100 cm., with thirteen elongated internodes, had been formed in the completely vegetative 15-hour series by November 17, and their apical meristems were still active. The few inflorescences produced in the 14-hour series, chiefly exserted in October, were all borne on long tillers with numerous internodes. In the 13-hour series, however, the photoperiodic stimulus to floral initiation became effective early in the development of each successively formed tiller, so that, once initiated, flowering in most

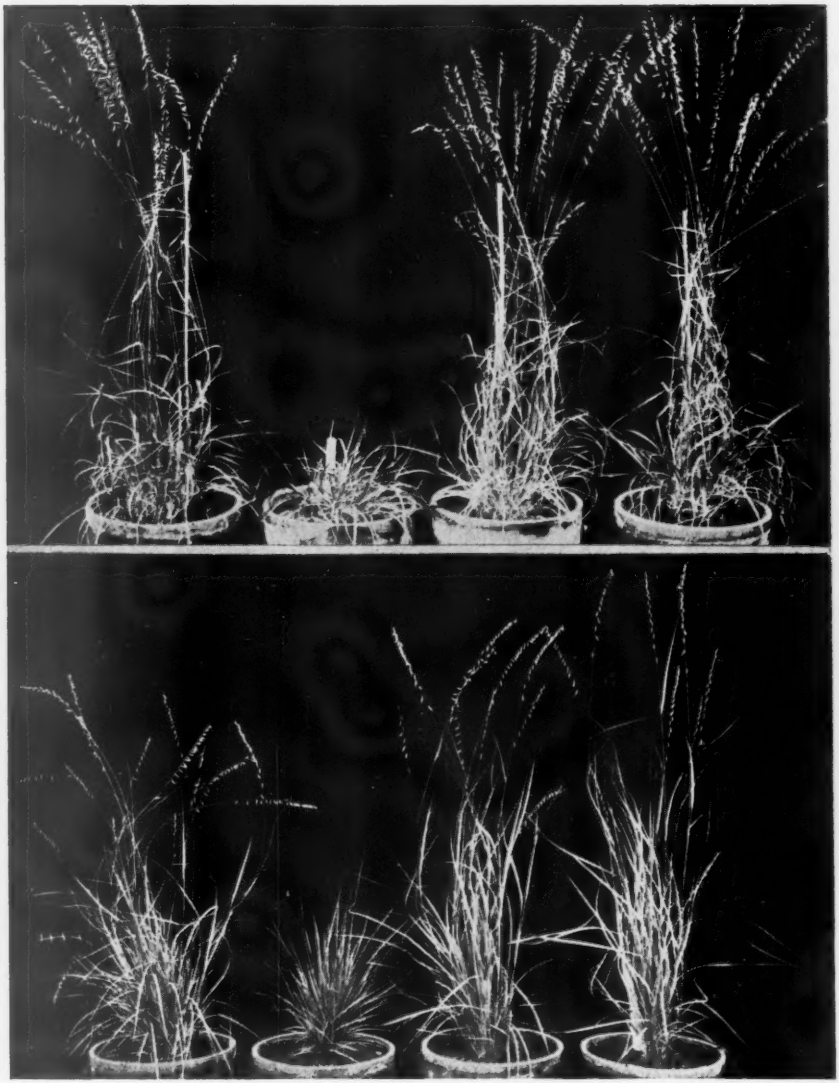
clones continued until late September or October, the last inflorescences appearing on October 20. Culms ranged 35–80 cm. in length and possessed three to five elongated internodes. They were almost twice as numerous as in the N series (table 3) but on the average were smaller in size. The great delay in flowering in the N series allowed the older culms to continue vegetative growth for a long time. Consequently, fewer tillers were produced than in the 13-hour series, where vegetative growth of each tiller was soon brought to a close by the initiation of an inflorescence primordium, and additional tillers were continuously forming and developing.

The cessation of inflorescence exsertion in the 13-hour series on October 20 may have been partly a consequence of decreased light intensity or other factors, since small young tillers, showing some internodal elongation, were present on November 17. They were apparently vegetative, although they were not dissected.

The variation in time of first exsertion and in duration of flowering by the various clones in the 13-hour series (tables 1, 2) was probably due largely to accidental differences in rate of establishment after transplanting, rather than to genetic differences affecting the rapidity of initiation on the 13-hour photoperiod.

FIG. 2.—Divisions of one clone of strain 1 (Texas), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 26, 1944. Division on natural photoperiod flowered vigorously from September 20 to October 13; division on 13-hour photoperiod exserted inflorescences from June 26 to October 6; the others failed to flower.

In summary, this population of strain 1 consists of intermediate- or short-day plants, somewhat similar in habit and response within each treatment of this experiment, with an upper critical photoperiod of approximately 14 hours. Any intermediate-day plants also have a lower critical photoperiod between 9 and 13



FIGS. 3, 4.—Divisions of two clones of strain 12 (North Dakota), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 26, 1944. Clone in fig. 3 (above) represents extreme in limitation of vegetative growth on 13-hour photoperiod. Most clones of this strain more closely resembled the one shown in fig. 4 (below) in both vegetative and reproductive habits, although its leaves in the 13-hour series are somewhat more erect and longer than the average.

hours. In their native environment in southern Texas they would seem to be adjusted by photoperiodic requirements to a long season of vigorous vegetative growth during the long days of spring and early summer, but they are delayed in flowering until late summer. Seed is ripe, however, long before any danger of frost in November. No data are available to the writer on the growth habits of this strain in the autumn months in southern Texas. The experiments indicate that photoperiod would not be a limiting factor of significance for vegetative growth in the autumn, or even winter months, since plants remain partly green and grow somewhat in the even shorter days of winter at Chicago, and on experimental 9-hour photoperiods in summer. The onset of dormancy or limited growth of this strain in Texas in autumn is thus probably not related to decreasing photoperiod, unless following as a consequence of the reproductive activity induced in part by this factor. By reason of this type of adjustment to the temperature and photoperiodic conditions in southern Texas, similar strains, when planted in more northern latitudes, continue vigorous vegetative growth on the long summer days (more than 14 hours) until August or September, may fail to ripen seed before autumn frosts, and are often winter-killed (7), in contrast with strains (such as strain 12) well adjusted photoperiodically to the northern conditions.

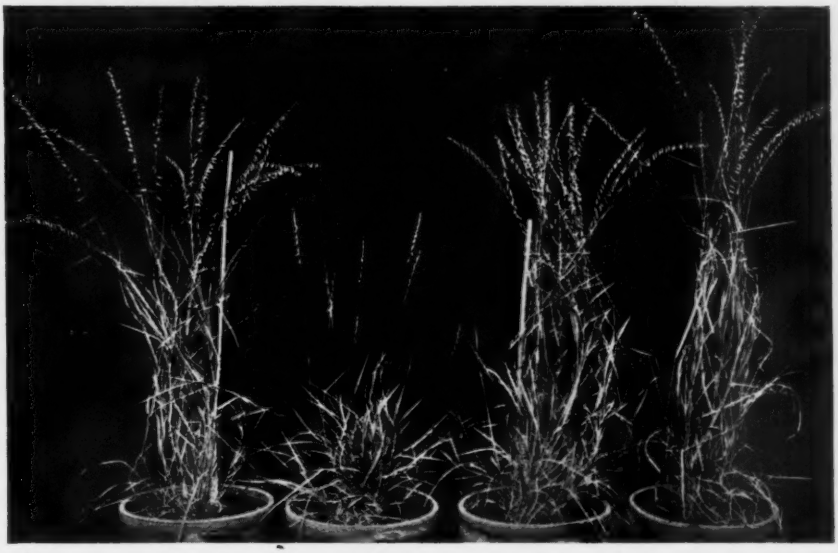


FIG. 5.—Divisions of one clone of strain 12 (North Dakota), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 26, 1944. This clone showed the maximum flowering response in this strain on 13-hour photoperiod (and approximately maximum vegetative growth on this treatment).

STRAIN 12.—The individuals of this strain from North Dakota showed greater variability than did those of strain 1, although not so much as did those of strain 5, both in flowering responses (tables 1, 2, 3; fig. 1) and in vegetative habit (figs. 3–5). Note especially the

variation shown in the 13-hour series. However, it is obvious from the data and from their appearance that all the selected individuals are intermediate- or long-day plants, with a short critical photoperiod for both vigorous growth and flowering between 13 and 14 hours. While more than half the clones were eventually able to initiate and exsert one or more inflorescences in the 13-hour series, the plants were small and the inflorescences few, weak, sterile, and exserted later in each clone, in contrast with the other three series (figs. 3–5; table 2). This critical photoperiod is approximately of the same length as the minimum natural photoperiod to which this strain is exposed in its growing season in North Dakota.

It is also of interest that the last inflorescences were exserted in the N series on August 30. These were probably initiated in late July or early August on decreasing photoperiods considerably in excess of 14 hours. Since flowering was as vigorous on 14- and 15-hour photoperiods as in the N series (table 3), and was much longer continued (fig. 1; table 2), the reason for the cessation of exsertion in the N series on August 30 is not entirely clear. It is possible that inflorescences were initiated in August in some plants which were not exserted because of the effect of photoperiods under 14 hours in limiting internodal elongation. Effective photoperiods decrease to this value in late August and early September at Chicago. Several of the clonal divisions in the N series became semidormant at this time, while others were still producing a few short tillers. In the former, dormancy may naturally follow as a consequence of a vigorous reproductive phase, irrespective of photoperiodic conditions, since the divisions of some of these clones in the 14- and 15-hour series ceased activity at about the same time (fig. 1). It is also possible that other external or internal factors may have been responsible. This is suggested by data on inflorescence number, culm length, and internode number. These values were approximately the same in the 14-, 15-hour, and N series for those culms elongating in June, July, and August. These are the culms visible in figures 3–5. They ranged from 55 to 80 cm. long, averaging about 70 cm., with four or five elongated internodes in all cases. The few flowering culms in the 13-hour series were much shorter (35–60 cm.), and some had only three elongated internodes. Those culms in the 14- and 15-hour series which elongated in September and October were shorter than those appearing earlier, ranging from 40 to 50 cm., but with the same internode numbers of four or five. The inflorescences were also smaller. Thus, while there was still a stimulation to activity in the autumn in these two series in contrast to the N series, in spite of similar vigorous flowering previously, it was not so effective as it had been, as shown by shorter internodes, smaller inflorescences, and a decrease in the rate of production of new inflorescences. While continued activity was probably stimulated chiefly by the long photoperiods, it is not clear what factors were responsible for the diminution of flowering in these two series. It does seem possible, however, that, in addition to these unknown factors, decreasing photoperiod did play a part in the onset of dormancy in the N series, which led to a practical cessation of both vegetative and reproductive activity in all clones, while some of those on longer photoperiods were still active. Plants of this strain, however, did grow very weakly on a 9-hour photoperiod in the previous experiments. It is possible that the effects of decreasing

short photoperiods are somewhat different from those of constant ones.

All these clones in the N series were able to initiate and exsert inflorescences on the longest days of June and July (effective photoperiods of 16 hours or more), although exsertion began slightly earlier in the 14-hour series than on natural or 15-hour photoperiods. The divisions of most individual clones, however, began flowering less than 10 days apart in these three series. Since numerous plants flowered on 20-hour photoperiods in previous experiments, it would seem that, if any of the individuals in the present experiment are intermediate- rather than long-day plants, their upper critical photoperiod is probably above 16 hours and thus of little practical significance in regulating development in their native environment.

The data indicate that these plants are well adjusted to the range of photoperiods in the frost-free growing season in North Dakota. After resuming growth in late April or early May,[2] they can initiate inflorescences as soon as they attain sufficient size. They may continue to flower—so far as photoperiodic restriction is concerned—until such time as the inhibiting effect of photoperiods decreasing to a certain level, and other factors, lead to the limited growth and onset of dormancy in late August and early September, which enables them to escape injury from the first autumn frosts of mid- or late September. According to Mr. ROGLER, seed is usually ripe at Mandan by August 20, and the plants are completely dormant by September 15, 10 days before the average date of the first killing frost. In so far as photoperiodic responses control the developmental cycle, these plants are thus well adjusted for seed production to the long photoperiods of the relatively short frost-free season of their native environment. They might produce more forage if they had an upper critical photoperiod of such length as to cause a delay in flowering until later in the summer, but they would then be less well adjusted to escape early frosts. ROGLER (7) classes side-oats grama as a "warm-temperature" grass in North Dakota, since it is dormant in the cool spring and fall months, during which "cool-temperature" grasses show active growth. This is in accord with its probable evolutionary history, with origin in low latitudes (6). The strain in North Dakota has evolved to become well adjusted photoperiodically to that latitude, while apparently retaining the same minimum temperature requirements for growth shown by more southern strains (7). It can, however, well endure winter temperature conditions unfavorable for southern strains.

STRAIN 5.—This strain showed considerable variability in vegetative habit and in flowering among the various clones (figs. 6–10). In general, the data confirm the conclusions based on nonidentical populations in the previous experiments—that this strain consists of both intermediate- and long-day plants. Flowering was less vigorous than in strains 1 and 12, although the plants grew well vegetatively on all treatments. For the strain as a whole the data do not show a well-defined uniform critical photoperiod, and the range of photoperiodic conditions of the experiment apparently controlled development less decisively than in the other two strains.

All clones except three eventually flowered on all four treatments. For the majority the long photoperiods in the 15-hour and N series apparently caused

[2] Data on growth in North Dakota from personal correspondence with Mr. GEO. A. ROGLER, Northern Great Plains Field Station, Mandan, N.D.

some delay in the exsertion of inflorescences, which were also few in number, in contrast with the development in the 13- and 14-hour series (figs. 1, 6, 7; tables 1, 3). Apparently correlated with the delay in flowering was a greater number of internodes per flowering culm on the longer photoperiods (N and 15-hour). Three clones were delayed in flowering in the N series until the first week in September. All clones except one flowered first in the 13- or 14-hour series. Three clones failed to flower on 15-hour photoperiod. One (fig. 9) of these three flowered on the other three treatments, another only in the 13-hour series (fig. 10), and the third in the 14-hour and N series. The failure of the first of the three to flower in the 15-hour series was most probably due to unfavorable photoperiod, since it flowered vigorously in both 13- and 14-hour series, and eventually in the N series. This clone is thus an intermediate-day plant whose upper critical photoperiod lies between 14 and 15 hours. The flowering responses of the clone which flowered weakly only on 13-hour photoperiod (fig. 10) was probably limited by genetic conditions rather than external ones, although a short-day plant with a critical photoperiod between 13 and 14 hours might be able to ripen seed in the native environment of this strain. Little emphasis should be placed on this clone or on the one which flowered only on 14-hour and natural photoperiods. Flowering in the latter was relatively weak in both treatments. The four inflorescences appearing in the N series were exserted between July 22 and August 16, and thus they must have been initiated on photoperiods of 15 hours or more. It is unlikely that failure of this clone to flower in the 15- and 13-hour series was due to unfavorable photoperiods.

Average number of inflorescences per plant was the same in the 13- and 14-hour series (table 3). A higher percentage of the clones exserted them simultaneously in these two series than in the other two (fig. 1), although the flowering seasons were more prolonged in the 14- and 15-hour series (to November 3) than on natural or 13-hour photoperiods (to October 6). It is not clear why exsertion ceased in the 13-hour series on this date. A few vegetative tillers were still being produced and growing, although with limited internodal elongation. By November 17 this series was mostly dormant, in contrast with continuing limited growth of some clones in the 14- and 15-hour series. The relatively vigorous early flowering may have hastened the onset of dormancy in the 13-hour series, especially if some of these clones have a lower critical photoperiod for vigorous growth and flowering near but somewhat below 13 hours. In previous experiments there was no flowering on 9-hour photoperiods, and vegetative growth was weak in comparison with the 13- and 16-hour series, although less so than in strain 12.

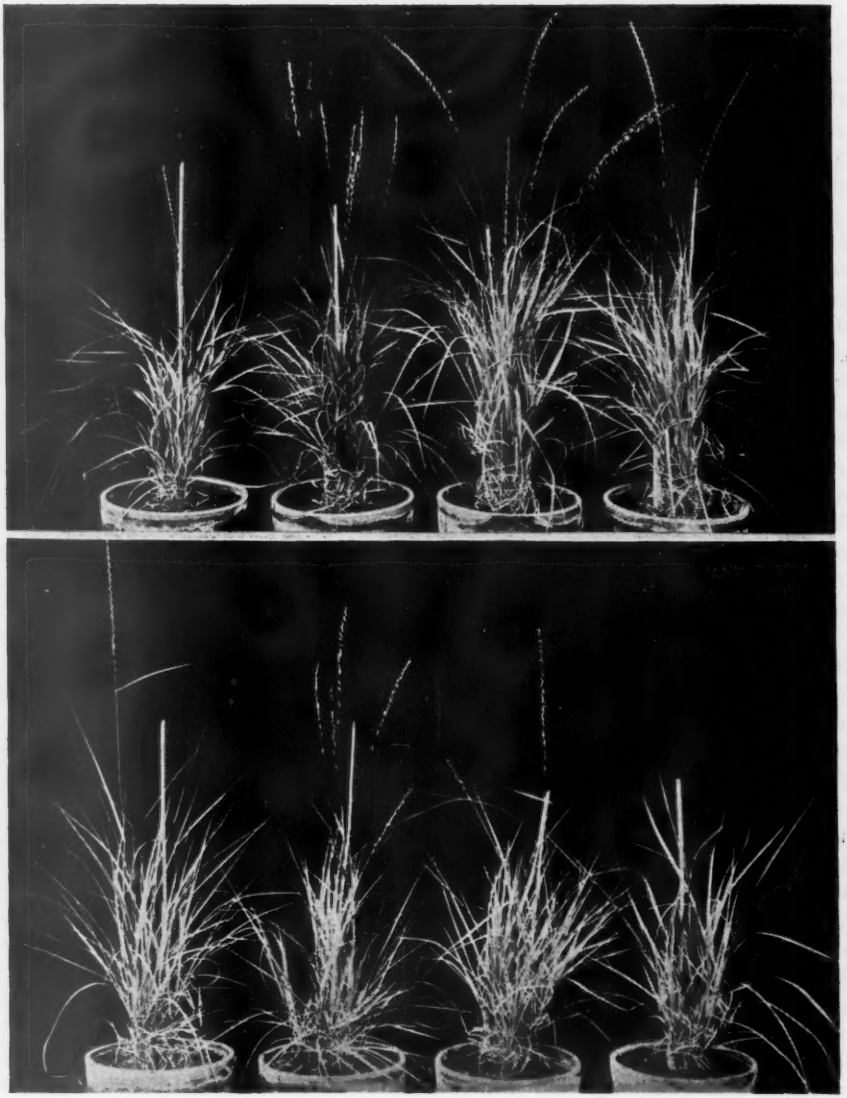
If flowering ceased in the N series in the present experiment partially as a consequence of unfavorable photoperiods, the last inflorescences to appear were probably initiated in early September on photoperiods about $13\frac{1}{2}$ hours long. However, some clones ceased flowering in mid-August. Their inflorescences must have been formed during the long days of July. Since these clones also flowered well on 13-hour photoperiod, their cessation of flowering in August in the N series does not suggest that it was due to decreasing photoperiods. In previous experiments some plants of this strain flowered on 16- and 20-hour photoperiods.

Most clones were in flower simultane-

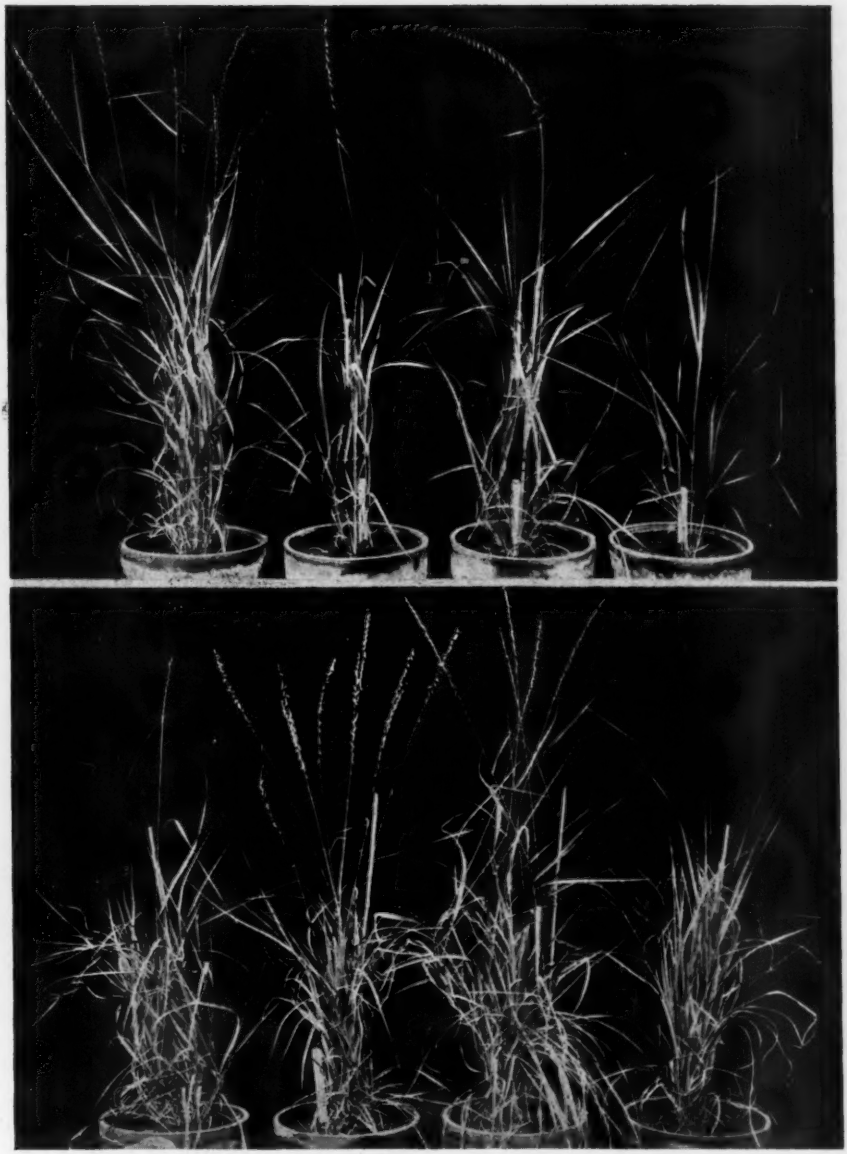
ously, at least for a time, in the 13- and 14-hour treatments, in comparison with their seeming differentiation into "early" and "late" individuals in the N and 15-hour series. This resulted from the considerably greater delay in flowering of some clones in the last two treatments and suggests that still longer photoperiods might have resulted in a differentiation into flowering and nonflowering individuals. If this should prove to be the case, the population would thus contain some individuals with an upper critical photoperiod, in addition to the one clone previously mentioned, although the length of this photoperiod might vary from plant to plant. Since no plants have flowered on 9-hour photoperiods, it seems reasonable to assume that all the individuals have a lower critical photoperiod between 9 and 13 hours, and thus none would be short-day plants. As in strain 12, this short critical photoperiod might lie near the lower limit of the photoperiodic range in which these plants are active in their native environment. Those plants with an additional upper critical photoperiod would be intermediate-day plants, while those able to flower on 20 hours are probably long-day plants. So far as photoperiodic conditions control their development, the latter should be able to flower at any time during the relatively long growing season in Oklahoma when they have attained sufficient size, and they are thus probably the "early" ones; while those with an upper critical photoperiod near 15 hours, or which are delayed in flowering on a 15-hour as compared with a 14-hour photoperiod, might well be delayed until later in the season. This strain begins to grow at Manhattan, Kansas, about April 20, usually exserts its first inflorescences about July 1, and reaches its peak of flowering about July 12.[3] In the Soil Conservation Service Nursery at Manhattan, where this has proved to be a highly desirable strain, a crop of seed is usually harvested in early August. If conditions remain favorable a second crop is produced in September, which is harvested in October. It would be possible, so far as photoperiodic requirements indicated by the present experiments are concerned, for many plants to contribute to both seed crops, while others might be so delayed in flowering on the long days of midsummer as to fail to reach maturity before early August but could produce seed for the October harvest. The latter type of plant, if it occurs, would probably produce the greatest amount of forage under field conditions, especially in wet years, since its vegetative growth would not be limited as a consequence of the early onset of reproductive activity. Under natural conditions this variability in photoperiodic requirement should lead to a long flowering season, with a greater likelihood of some seed production each year than if all plants matured at nearly the same time, as they apparently do in strains 1 and 12.

The available data do not show whether the onset of dormancy in this strain is hastened by decreasing photoperiods, although this is possible, or whether vegetative growth is much limited by this factor during the late part of the growing season in the Oklahoma environment. At Manhattan, Kansas, it is dormant by October 10, approximately the average date of the first killing frost. Some plants in the N series were still growing slightly and their green foliage ranged 30–60% on November 17, when the natural photoperiod at Chicago had decreased to a level below that at which this strain is

[3] Personal correspondence with Mr. D. R. Cornelius, Soil Conservation Service Nursery, Manhattan, Kansas.



FIGS. 6, 7.—Divisions of two clones of strain 5 (Oklahoma), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 27, 1944. Note contrasts in vegetative habit among clones shown in figs. 6–10. Clone in fig. 6 (above) is a long-day plant, although it flowered first and more vigorously on 13- and 14-hour photoperiods. Clone in fig. 7 (below) flowered on 15-hour photoperiod in September.



FIGS. 8, 9.—Divisions of two clones of strain 5 (Oklahoma), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 27, 1944. Clone in fig. 8 (above) flowered on 15-hour photoperiod in October. Clone in fig. 9 (below) is an intermediate-day plant, with upper critical photoperiod between 14 and 15 hours, and was considerably delayed in flowering on natural and 14-hour photoperiods in comparison with the 13-hour division.

normally subjected to frost in Oklahoma. In contrast, however, 50–80% of the foliage in the 15-hour series was still green on this date, with slightly less amounts in the 14-hour series. It is possible that the greater longevity of foliage in the latter two series is related to the artificial light which they were receiving when natural light intensities had decreased to low values, and not to the effects of longer photoperiods. Both factors may be responsible, since the 13-hour series showed death of foliage comparable with the N series.

FIG. 10.—Divisions of one clone of strain 5 (Oklahoma), grown after April 16, 1944, on photoperiods of (left to right): Chicago natural daylength, 13, 14, and 15 hours. Photographed August 27, 1944. This clone is strongly vegetative on all treatments and is not classified photoperiodically.

In summary, this strain is highly variable in both vegetative (figs. 6–10) and reproductive habits. All individuals tested apparently have a lower critical photoperiod between 9 and 13 hours, while one of the experimental plants likewise has an upper critical photoperiod between 14 and 15 hours. This clone, and several which were considerably delayed in flowering on 15-hour photoperiod and on the long days of summer at Chicago—in contrast with development in the 13- and 14-hour series—are probably intermediate-day plants. Other individuals, able to flower on 20-hour photoperiods, are probably long-day plants, although the data of 3 years indicate some delay in exsertion of inflorescences on such long photoperiods in contrast with 13-hour treatments. This variability in photoperiodic response should make it possible to acclimatize certain selections from this strain to areas considerably north or south of its native latitude, so far as photoperiodic limitations are concerned.

VEGETATIVE GROWTH

The present experiment adds information to one point which merits attention. In the previous experiments it was shown that vegetative growth of strain 12 on 13-hour photoperiod was very limited in contrast with behavior in the 16- and 20-hour series. Figures 3–5 indicate that the critical photoperiod for such severe limitation falls between 13 and 14 hours.

This is the more striking if one bears in mind that these series differed by only one hour of artificial light of low intensity, each receiving the same 9 hours of natural daylight. While such sharp limitation is often found in long-day plants, the contrast is very great in this strain. Whatever the mechanism physiologically, for vigorous growth this strain apparently requires a photoperiod as long as the minimum to which it is exposed in its natural growing season.

Such sharp differences in vegetative growth over a narrow range of photoperiods have not been indicated in strains 1 and 5. The differences among treatments in the present experiment are related primarily to the effect on reproductive activity of the different photoperiods, with correlated gross effects on vegetative growth and habit, and not obviously to an influence of photoperiod expressed directly on leaf size, internodal elongation, and other vegetative characters. While a critical photoperiod for flowering in strain 5, and for some plants of strain 1, lies between 9 and 13 hours, differences in vegetative habit between these two treatments in each strain are less than between the 13- and 14-hour series of strain 12. A sharp photoperiodic limit below which vegetative growth is severely limited should thus not be expected in strains 1 and 5, at least between 9 and 13 hours of light per day. This is in general accord with the responses of many short-day plants adjusted to the photoperiodic conditions of low latitudes, such as strain 1. Thus, while strain 5 contains intermediate- and long-day individuals so far as flowering responses are concerned, in some respects its vegetative habits in response to a range of photoperiods are more like strain 1 than 12. It is thus suggested even more strongly than its latitudinal position would indicate that strain 5 does represent a transitional condition between strains 1 and 12.

Discussion

Many points developed at length in the preceding paper (6) will not be repeated here, and reference is made to the "Discussion" in it for conclusions additionally confirmed by the new data. The present experiment on clonal divisions of the three strains does confirm most of the conclusions previously based on nonidentical populations, as to the nature of the photoperiodic responses, and indicates more precisely the length of critical photoperiods for selected individuals in each strain, the diversity within strains, and the practical importance of photoperiod in the seasonal and latitudinal adjustment of the strains.

The selected individuals of strain 1 (Texas) showed less diversity than did those in strains 5 (Oklahoma) and 12 (North Dakota) in all respects. Strain 5 is more variable than strain 12. FULTS'S data (3) suggest that strain 1 lies east and south of the present center of greatest genetic and cytological diversity of the species in the United States, whereas strain 5 is native in this center, and strain 12 is considerably north of it.

All the tested individuals of strain 1 from southern Texas are intermediate- or short-day plants, with an upper critical photoperiod for rapid and vigorous flowering between 13 and 14 hours. Some clones flowered weakly after long delay on 14-hour photoperiod. The selections included one plant which had previously flowered weakly on 16-hour photoperiod after 8 months of growth but did not flower on 15-hour photoperiod in this experiment. While the strain may include other individuals eventually able to flower on such long photoperiods, from the standpoint of regional adaptation they

apparently are all short-day plants which would probably be unable to ripen seed if planted very far north of their native latitude, although they are able to produce luxuriant vegetative growth in their first year on the longer days of northern latitudes. They suggest that long-day individuals or intermediate-day ones with a longer critical photoperiod might evolve from this strain in a slow northward migration. In its native environment, photoperiod undoubtedly exerts some control over the developmental cycle by prolonging vegetative activity and causing some delay in the initiation of inflorescences, but probably it does not directly influence dormancy or limit growth in the autumn. This strain is still non-rhizomatous under the experimental conditions, in contrast with strains 5 and 12.

In the North Dakota strain the new data strikingly confirm a long-day status. The critical photoperiod lies between 13 and 14 hours, below which vegetative growth is very much limited, with little internodal elongation, although some clones flowered weakly. Inflorescence initiation probably occurs rapidly on photoperiods above this level in June and July in North Dakota, while the onset of dormancy in late August and September coincides with the decrease of photoperiod below the critical. Thus this strain is active in North Dakota for about a 4-month period and is completely dormant before danger of frost. If planted too far south of its native latitude, in areas where daylengths exceed its critical for only the few weeks centering on the summer solstice, it would show very limited growth and flowering in comparison with more southern strains.

Strain 5 from central Oklahoma shows much more variability and consists of both intermediate- and long-day plants, with a lower critical photoperiod below 13 hours. Vegetative growth is vigorous on photoperiods of 13 hours or more. The intermediate-day plants probably vary in the value of the upper critical photoperiod. In one individual it lies between 14 and 15 hours. Such variability should lead to a long flowering season, and, so far as photoperiodic conditions are concerned, to relatively easy acclimatization of selections from this strain to areas north or south of its native latitude.

The short-day condition of strain 1 was probably the original photoperiodic response in the species, as pointed out previously (6), since the species undoubtedly spread to the north after an origin in low latitudes—where this response is now typical. This would have involved the evolution of the intermediate- and long-day responses as the migration occurred. If crosses between the different strains are possible, a photoperiodic study of the parental, F_1, and F_2 generations might yield data on the possible evolutionary mechanism of such adjustment, as suggested by the recent work of GOODWIN (4). In conjunction with fundamental studies on the physiology of photoperiodism in the various strains, including vegetative as well as flowering responses, it might be possible to ascertain whether the differences between short- and long-day plants are absolutely qualitative or are primarily quantitative in nature. The existence of both types in one species, linked by transitional ones (such as occur in strain 5), suggests that the qualitative differences between the types are the visible expression of only quantitative differences in the physiological mechanism which induces flowering.

Summary

1. Clonal divisions of twelve selected individuals of each of three strains of side-oats grama were grown from April

16 to November 17, 1944, on Chicago natural daylength and on photoperiods of 13, 14, and 15 (or more) hours. The three strains from Texas (San Antonio), Oklahoma (El Reno), and North Dakota (Cannonball) represent the extremes and approximate means of latitudinal origin and range of response of twelve strains previously grown over a 2-year period on 9-, 13-, 16-, 20-hour, and natural photoperiods. The selected individuals sampled the diversity of each strain.

2. All clones from Texas flowered rapidly and persistently on 13-hour photoperiod, and in late September on natural photoperiod. Some flowered weakly in September and October in the 14-hour series. None flowered in the 15-hour series. Vegetative growth was vigorous on all treatments. These clones are intermediate- or short-day plants with an upper critical photoperiod for rapid and vigorous flowering between 13 and 14 hours. All clones from North Dakota grew and flowered vigorously on natural, 14-, and 15-hour photoperiods. On 13-hour photoperiod growth was very limited, although some clones flowered weakly. These clones are long-day plants with a critical photoperiod for vigorous growth and flowering between 13 and 14 hours. Most clones from Oklahoma flowered equally well and fairly rapidly on 13- and 14-hour photoperiods. Most of them eventually flowered on natural photoperiod, but were delayed, in contrast to the 13- and 14-hour series. Most of them either failed to flower or were delayed in flowering on 15-hour photoperiod. These data and previous observations indicate that this strain includes both intermediate- and long-day plants, both types having a short critical photoperiod between 9 and 13 hours. One of the former has an upper critical photoperiod between 14 and 15 hours.

3. Such strong photoperiodic differentiation and adjustment have seldom been reported within a single native species. The series of photoperiodic types within it raises interesting questions as to the evolution and nature of the responsible genetic and physiological mechanisms, since the species probably originated in low latitudes, where the short-day response is typical, with subsequent adjustment to the longer days encountered in the northward extension of its range. Other implications of the findings discussed in this or the preceding paper (6) concern some of the causes of the morphological diversity among the latitudinal strains when grown together, as reported by other workers, and the bearing of photoperiodic adjustment upon possible acclimatization of strains to latitudes lower or higher than their native ones.

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THE GAMETOPHYTE OF DIPTERIS CONJUGATA

ALMA G. STOKEY

Introduction

The genus *Dipteris* has been a taxonomic problem ever since REINWARDT established it in 1824 with the species *D. conjugata*, which had previously been placed in the genus *Polypodium*. Several of the later pteridologists of the nineteenth century returned *D. conjugata* (and its related species as they were described) to the genus *Polypodium*, on account of the naked sorus. DIELS (8) placed the genus *Dipteris* in the Aspideae, stating that it is an isolated type but in vegetative characters has more in common with the Aspideae than with other groups. CHRISTENSEN (5) in 1906 gave it the same position as DIELS. SEWARD and DALE (10), however, in 1901 had approached the problem from the morphological and paleobotanical side and met the difficulties by the establishment of a new family, Dipteridineae (Dipteridaceae). Since then the sorus of three species has been investigated by ARMOUR (1), who considered *D. conjugata* the most advanced of the three in soral characters. The ontogeny of the stele in two species, including *D. conjugata*, was investigated by DE BRUYN (7). BOWER (2) in 1915 discussed *Dipteris* and its probable derivatives in connection with the study of *Cheiropleuria bicuspis*, and again (3) in 1926, with reference to its phylogeny as indicated by anatomy, leaf architecture, sorus and sporangium—emphasizing (as did SEWARD and DALE) its similarities to *Gleichenia* and *Matonia*. BOWER accepted the family Dipteridaceae, and this position has been taken in several recent works treating of the phylogeny of ferns; although as late as 1938 Christensen (6) retained *Dipteris* in the Polypodiaceae, assigning it to the subfamily Dipteridoideae between the subfamilies Dryopteridoideae and Polypodioideae. It has been pointed out, in considering the position of *Dipteris*, that a weak point has been our lack of knowledge of the structure and development of the gametophyte. We have had only the figure shown by BOWER (3, p. 318) of a mature gametophyte collected by Dr. LANG on Mount Ophir, Malay Peninsula.

Material and methods

The spores of *Dipteris conjugata* Reinw. were obtained from fertile leaves collected by the writer from plants growing in the Botanical Garden at Tjibodas, Java. Although plants were seen growing wild in the mountains, they were much less accessible. The plants in the garden were growing in large clumps and were vigorous, healthy, and fruiting abundantly. The first collection and cultures were made in 1931, but the cultures did not live long enough to develop late stages of the gametophyte. On a second visit to Tjibodas in 1937, collections of spores were again made and cultures started. The spores were planted within a few hours after they were collected; they lost their viability quickly and would not germinate when more than a few days old. The early stages were studied in the Treub Laboratorium, Buitenzorg; then the cultures were brought to the United States and work continued at Mount Holyoke College and the Marine Biological Laboratory, Woods Hole, Massachusetts. The gametophytes are still in culture. Germination stages were obtained on distilled water

and on peat, and the later stages on peat by methods described in a previous paper (11). Fresh gametophytes were used for the study of germination stages, habit of growth of later stages, distribution and external aspects of sex organs, and habit of growth of apogamous plants. Sections were used for internal structure; for killing and fixing, a weak to medium chromo-acetic acid solution and a modified Navashin's solution were used.

Observations

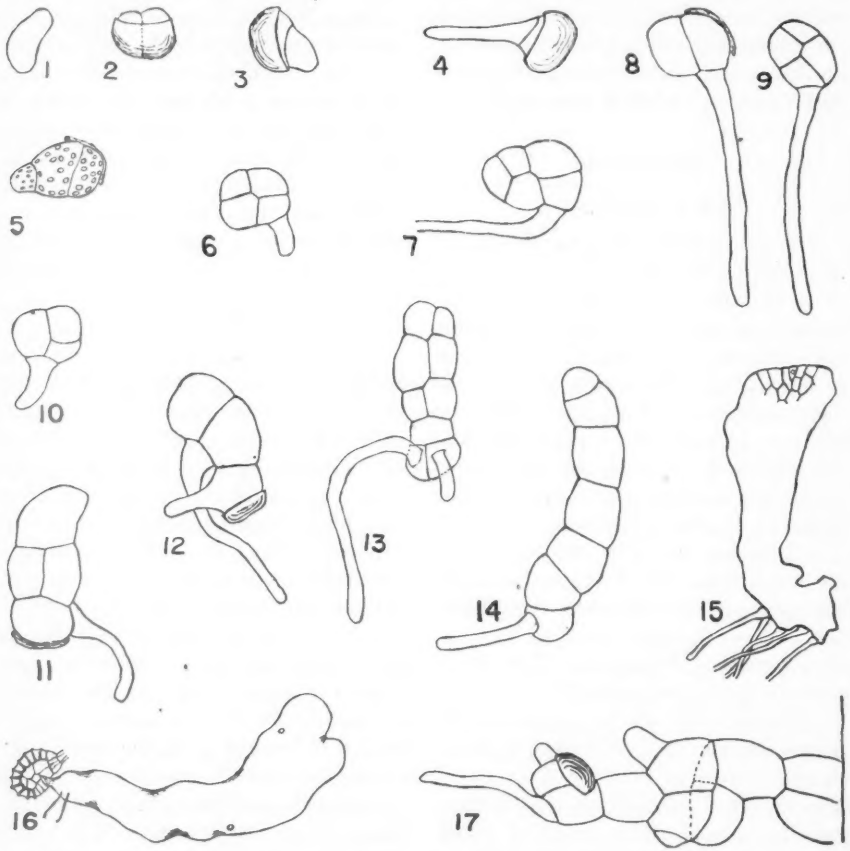
Spore germination

The spores of *D. conjugata* are bilateral, varying in size from 18 × 35 to 21 × 44 μ (fig. 1). Seward and Dale described the spores of *Dipteris* as bilateral, but Christensen (6) has described them as tetrahedral. The spores are pale, almost colorless, with a smooth thin coat without perispore, and appear whitish in the mass. A considerable number of sterile spores without granular contents appeared in the collections. The sporangia do not discharge effectively, and some spores are left in the sporangium. The spores are less powdery than in the case of most ferns and tend to stick together, making it unusually difficult to get well-distributed prothalli in cultures; accordingly, the spores germinate in groups, and this tends to make the gametophytes irregular in form. There were often detached sporangia in the cultures, and the spores which remained inside germinated readily. The germination period was about 2 weeks, which is rather slow. The first division of the spore was usually perpendicular to the long axis, and one of the two cells usually produced a rhizoid (figs. 2, 5, 6), although a rhizoid arose as a result of the first division in some cases (figs. 3, 4, 8). In most cases a plate was formed, or less frequently a more or less irregular mass, with great variation in germination stages (figs. 7, 9–13). In this material germination seldom resulted in a filament, and if one was formed—as in the case of germination in a sporangium (fig. 14)—the tendency to form elongated cells was weak or lacking. When a spore germinated in the sporangium the prothallus usually broadened by lengthwise divisions in the filament as soon as the anterior portion became free (fig. 16). Considerable enlargement of the cells of the plate usually preceded elongation of the thallus. In the early stages the gametophytes grew very slowly and in 4 weeks had formed not more than four to six cells and a rhizoid.

Gametophytes

After the early stages, the development of the young gametophyte is that of the usual type found in leptosporangiate ferns, with growth from a single apical initial (fig. 15) followed by growth from a group of initials and formation of a heart-shaped prothallus with midrib. The form of the young regenerated gametophytes in figure 18 gives the type of those arising from spores. If conditions are unfavorable and the thallus is crowded, it may assume a ribbon-like form with a cordate apex (fig. 16). The rhizoids are pale brown in color and are usually limited to the region of the midrib, but in old gametophytes the rhizoid region is not always continuous along the rib. Many chloroplasts were found in young rhizoids, particularly in the case of young gametophytes. Under some conditions there is considerable irregularity in the development of the plate and the region at the base of the thallus (fig. 17). In cultures 4–5 months old there were many small gametophytes, consisting of relatively few cells, frequently with irregular bases, suggesting an asymmetri-

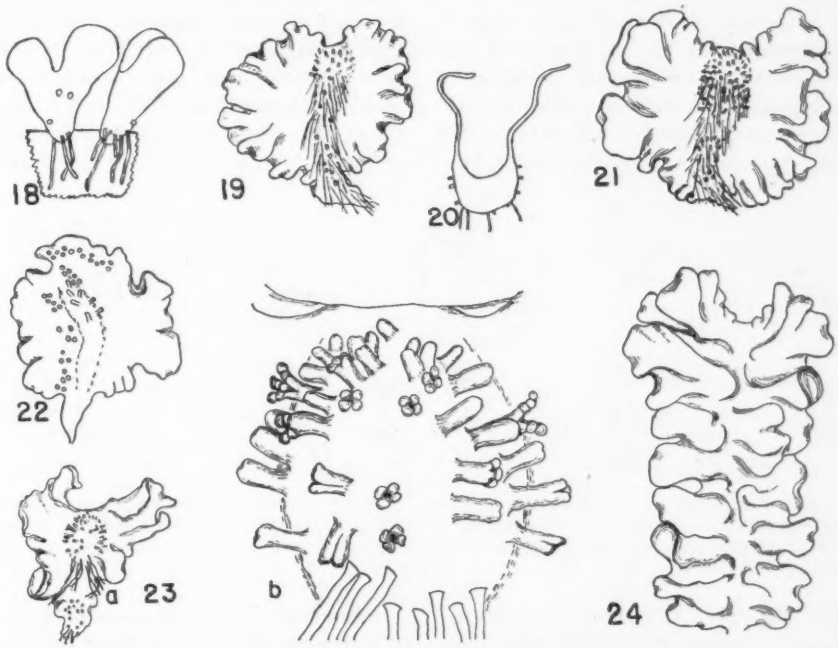
cal type of germination as a mass. Even in the irregular gametophytes there were few which had filamentous portions. The cultures were in transit from Java to the United States during the early stages of development, and although every effort was made to keep them in suitable light with little change of direction, the exigencies of travel may be responsible for some of the irregularities. In the case of the gametophytes of most ferns, however, the usual effect of unfavorable conditions is to cause them to become filamentous, but this rarely happened in *D. conjugata*. Greater irregularities were found in this species than in the various others subjected to the same conditions.



FIGS. 1–17.—Fig. 1, spore. Figs. 2–14, early stages of gametophyte. Fig. 15, gametophyte at apical cell stage. Fig. 16, gametophyte which has germinated in sporangium. Fig. 17, irregular base of young gametophyte.

The mature gametophytes have a heavy midrib and ruffled wings which may be more or less lobed (figs. 19, 21, 24). The top of the rib of an old thallus may be entirely concealed by the wings,

and the cross-section of the thallus may be like that in figure 20. The older parts of the thallus usually died away at a relatively early age, so that the gametophytes in these cultures never attained the great length of thallus which may be found in old gametophytes of *Osmunda*. The ruffled wings on the relatively short thallus often suggest a head of curly lettuce. In a few gametophytes a certain amount of starch was found stored in the midrib, as was reported for *Marattia sambucina* (12). In appearance the mature gametophytes resemble those of *Gleichenia*, as shown by CAMPBELL (4) in his figures of *G. pectinata*, *G. laevigata*, and *G. dichotoma*, and as found by the writer in several other species in culture. They show the same type of ruffled wings and heavy midrib, both in surface view and in section. The tendency of the midrib of old gametophytes to branch, which is not unusual in the Osmundaceae, Gleicheniaceae, Cyatheaceae, and Dicksoniaceae, was almost lacking in these cultures; only a few gametophytes gave even a suggestion of a forking of the midrib, and in no case was it long-continued. No hairs of any type were found on the gametophyte of *D. conjugata*, even on those old enough to have more than 100 archegonia. Aging gametophytes may show great vigor in the production of regenerated branches (fig. 18). Cultures



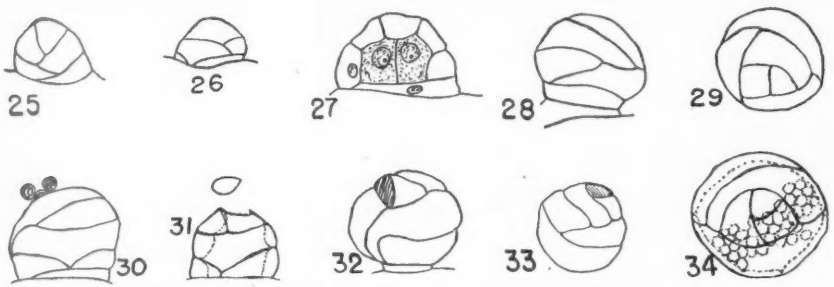
FIGS. 18–24.—Fig. 18, portion of old thallus with two regenerated gametophytes bearing antheridia. Figs. 19, 21, mature gametophytes bearing antheridia and archegonia. Fig. 20, cross-section of old thallus with archegonia and rhizoids. Fig. 22, gametophyte which bore first antheridia, then archegonia, then antheridia. Fig. 23: *a*, mature gametophyte with antheridia and archegonia; *b*, cushion of same thallus with archegonia and rhizoids. Fig. 24, old gametophyte about 7 mm. long, dorsal view.

which were started in July, 1937, are still flourishing after five or six transplantings—made possible in part by the abundant production of regenerated branches.

Reproductive structures

The mature gametophyte of *D. conjugata* is monoecious, but the production of antheridia is initiated before that of archegonia (figs. 18, 19, 21–23). If conditions cause a check in the formation of archegonia there may be a vigorous production of antheridia, not only on the wings but also on the cushion behind the apex (fig. 22). Many slender gametophytes bearing antheridia only are formed under conditions unfavorable for the large monoecious gametophytes, but they are not ameristic. The filamentous, branching, ameristic type of antheridium-bearing gametophyte frequently found in the Polypodiaceae was not found in these cultures. While both antheridia and archegonia are characteristically found on the ventral surface, both may be found on the dorsal. Old gametophytes with the midrib more or less concealed by the ruffled wings are especially likely to have archegonia on the dorsal surface, and irregular gametophytes are likely to have antheridia on the margin as well as on the dorsal side.

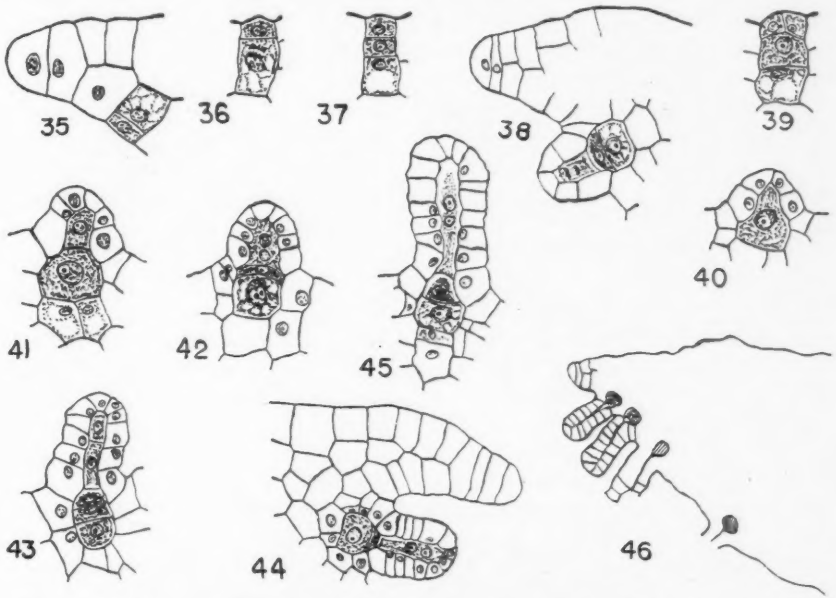
The antheridium is of the primitive and complex type found in *Osmunda* and *Gleichenia*. The wall consists typically of five or six cells, but there is considerable variation which seems to be related to the size of the antheridium. There is a wedge-shaped basal cell which usually does not extend completely across the antheridium (figs. 27, 30). Then there is a succession of oblique walls such as are found in *Osmunda* and *Gleichenia* (figs. 25, 26, 28). These cells, which are fairly regular at first, may become twisted and take peculiar forms with growth of the antheridium (figs. 32, 33). After the formation of the oblique intersecting walls a cap cell is formed which divides once or even twice (figs. 29, 34), forming a small opercular cell which is thrown off with the rupture of the antheridium (fig. 31). On young gametophytes the antheridia may be small but are rarely, if ever, as small as those of the Polypodiaceae. On well-grown gametophytes the number of sperms in an antheridium is large, and the antheridium is usually at least six to eight sperms in diameter, but it may be ten or eleven (fig. 34). The walls have a cutin layer which is particularly heavy



Figs. 25–34.—Figs. 25, 26, young antheridia. Fig. 27, section of young antheridium. Fig. 28, mature antheridium. Fig. 30, antheridium discharging sperms on side away from observer. Fig. 31, empty antheridium with opercular cell. Figs. 32, 33, empty antheridia, side and oblique views. Figs. 29, 34, top view of antheridia.

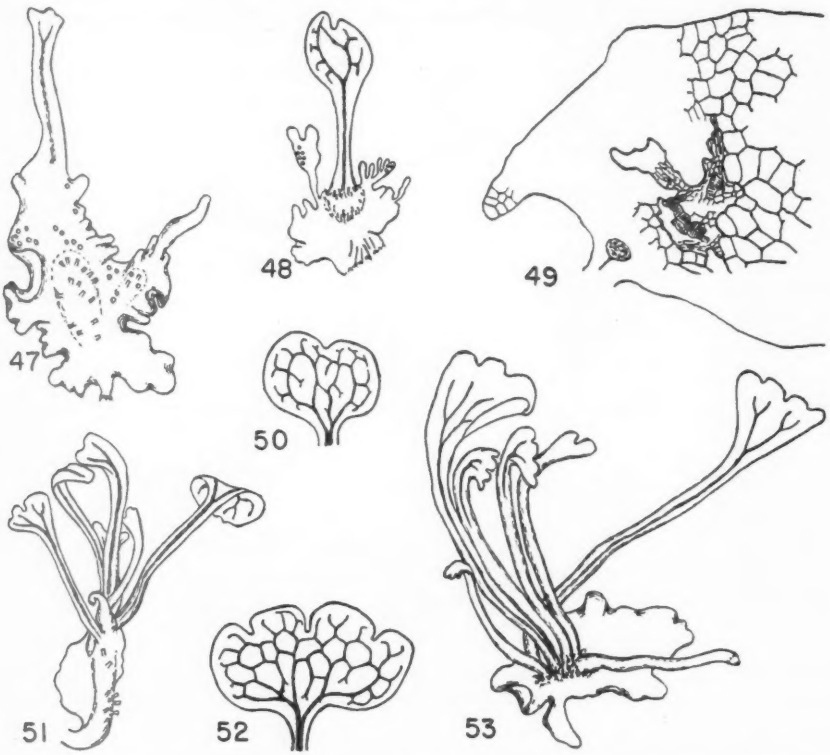
on the basal cell and the lower half of the antheridium. Swimming sperms were found abundantly in these cultures and were just as abundant on regenerated gametophytes as on those which arose from the spore.

The development of the archegonium conforms to that of the type found in leptosporangiate ferns. The initial usually arises close to the apex and the primary neck cell is soon cut off (fig. 35). The characteristic "row of three" is formed, and division occurs in the primary neck cell (figs. 36, 37, 39). There is a vigorous enlargement of the central cell as it pushes between the neck cells and then a wall cuts off the neck canal cell (figs. 40, 41). The axial row is completed long before the neck has attained its full number of cells (figs. 38, 42). At the stage shown in figure 42 the archegonium might be one of the Polypodiaceae, but in later stages the difference in the neck is evident. The neck is usually much longer than those characteristic of the Polypodiaceae, with usually seven or eight cells in each row, but nine is not unusual. The neck is not only long but the curvature is slight—if any (figs. 44–46). The impression received in examining fresh material of old gametophytes is of strikingly long necks, which, on the younger part of the thallus, are pointed forward or in a forward direction but on the older parts are directed downward or laterally and are most abundant on the flanks of the midrib, with relatively few in the center (fig. 23*b*). At the time of maturity of the archegonium, when it is ready to open and fertilization might be expected, the tip of the archegonium points in a forward direction, with its



FIGS. 35–46.—Fig. 35, tip of gametophyte with early stage of archegonium. Figs. 36–42, 44, 45, stages in development of archegonium. Fig. 43, abnormal archegonium. Fig. 46, longisection of old thallus.

long axis approximately parallel to the dorsal surface (fig. 44) or making an angle which is usually less than 30°. In most archegonia observed there was no difference in the number of cells on the anterior and posterior sides, although there was sometimes a difference in size of cells, especially at the base. The surface of the necks is usually more or less cutinized. Cases of anomalous structure of the archegonium were few. On one gametophyte one archegonium had two ventral canal cells and a wall between the neck canal nuclei (fig. 43), while another had two neck canal cells and two additional nuclei not separated by a wall. The formation of four neck canal nuclei, which is not unusual in the Cyatheaceae and Dicksoniaceae, did not occur in any of the material sectioned. Although archegonia were formed abundantly on both old and young gametophytes, and sperms were also abundant, no cases of fertilization were found and no embryos seen in sections of the gametophytes, nor any young sporophytes of even probable syngamous origin. Ordinarily in cultures where sperms are abundant one is likely



FIGS. 47–53.—Fig. 47, gametophyte bearing antheridia, archegonia, and apogamous leaf. Fig. 48, gametophyte with antheridial lobe and apogamous leaf. Fig. 49, longisection of gametophyte with xylem elements. Figs. 51, 53, gametophytes with apogamous sporophytes. Figs. 50, 52, young leaves of apogamous sporophytes.

to find at least a few embryos in sections and an occasional syngamous sporophyte in the cultures.

Apogamy

Apogamy occurred very freely in these cultures. The first apogamous structures appeared when the cultures were a year old, and production is continuing to the present time. In some cases, as in the 1-year-old gametophyte shown in figure 47, the apogamous growth arose as a gradual extension of a relatively thin thallus into a leaf with a well-defined vascular system. In other cases the transition was more abrupt, with the transformation of the apex into a thickened mass which bore glandular sporophytic hairs and continued into an apogamous leaf (fig. 48). As shown in figures 47 and 51, a gametophyte which has been bearing antheridia and archegonia may become apogamous. Marginal lobes bearing antheridia were found on gametophytes with apogamous leaves (fig. 48), and a gametophyte was found which was bearing an apogamous leaf as well as regenerated branches which had both antheridia and archegonia. Under favorable conditions a succession of leaves appeared on the apogamous growth and ultimately a stem and root or roots (figs. 51, 53). The first root was late in developing and usually appeared on the dorsal side of the gametophyte. The sporophyte shown in figure 51 had a slightly larger root on the dorsal side of the thallus. The first leaf showed the forking of the petiolar bundle characteristic of *D. conjugata*, and at an early stage there could be seen the anastomoses and network characteristic of the later leaves (figs. 48, 50, 52). Well-rooted sporophytes which were probably apogamous were transferred to soil, but none survived the perils of early growth beyond the leaf height of 7–8 cm. In only one thallus was there found the type of apogamy shown in figure 49—the development of xylem elements and small-celled sporophytic tissue in the midst of the large-celled gametophytic tissue of the cushion. In general, apogamous structures were more common on thin prothalli than on thick. The apogamous structures were never associated with abortive archegonia. Perhaps in these cultures apogamy might be regarded as obligate, since growth conditions or inherent habit did not favor the formation of syngamous embryos, although there was abundant production of archegonia and antheridia.

Discussion

The preceding account shows that the gametophyte of *D. conjugata* bears a much closer resemblance to the gametophytes of *Gleichenia* and the primitive ferns than to those of the Polypodiaceae. In the experience of the writer, gametophytes of the cordate type show little if any difference within the limits of a genus, particularly in small genera. In the case of a genus as small and as sharply defined as *Dipteris*, it is probable that the gametophyte of *D. conjugata* is representative of the genus.

Germination in *Dipteris* is of a primitive type, consisting as it does of the formation of a plate, occasionally of a mass, and rarely of a filament. The plate and mass type are found only in primitive families, such as the Marattiaceae, Osmundaceae, and Gleicheniaceae. The only published account of germination in *Gleichenia* is that of Rauwenhoff (9), who described two types—germination resulting in a mass and germination resulting in a filament, with considerable irregularity and variation in both. The writer has had a few species of *Gleichenia* in cultivation from time to time and has

found that plate and mass germination occur in some species, and that there may be also the formation of a broad base like that in the Cyatheaceae and Dicksoniaceae, but that the tendency to form a filament is much weaker than in the Polypodiaceae. The mature gametophytes of *D. conjugata* developed in culture show the same habit of growth as those from Mount Ophir—a notably thick midrib and wings with a high degree of ruffling. This is the type found in at least several species of *Gleichenia*. The antheridia are quite unlike those of the Polypodiaceae. They differ in their greater size, in the larger number of cells found in the wall, and in their large sperm output. The large output of the antheridium is in contrast with that of the *Dipteris* sporangium, which has an output of only sixty-four spores. The antheridia of the Polypodiaceae seem to be uniform in the number of cells in the wall, except for the few species which may have a divided cap cell. The antheridia of *Dipteris* suggest the type found in several families of primitive ferns, such as the Osmundaceae and Gleicheniaceae, and are more like those of the Cyatheaceae and Dicksoniaceae than those of the Polypodiaceae. The archegonium agrees in development with that of the Polypodiaceae, Gleicheniaceae, and other leptosporangiate ferns, but the mature archegonium is peculiar in its long straight neck. The straight neck is found in the Osmundaceae, Hymenophyllaceae, and in some of the Cyatheaceae, but in these families it is not usually so long as in *Dipteris*. It is more like the neck of the archegonium in *Gleichenia*, although in some species of *Gleichenia* the neck may be even longer, with a forward curve, but it may be straight.

Dipteris, then, allies itself with the more primitive ferns rather than with the Polypodiaceae in the characters of the gametophyte: germination, mature habit, structure and size of antheridium, and an archegonium with a long straight neck. The gametophyte of *Dipteris* has more in common with the Gleicheniaceae, with which it is considered to be allied in sporophytic characters, than with the Polypodiaceae, since it does not have the filamentous type of germination, the relatively thin thallus, the symmetrical antheridium with three wall cells and small sperm output, and the archegonium with the short recurved neck. The contribution of the gametophyte to the question of the classification of *Dipteris* indicates that *Dipteris* does not belong to the Polypodiaceae; it emphasizes the appropriateness of the family Dipteridaceae established by SEWARD and DALE on sporophytic characters.

Summary

1. Germination of the spore in *Dipteris conjugata* results in a plate, in a more or less irregular mass, or (more rarely) in a filament.

2. The mature gametophyte has a thick midrib and ruffled wings; it is similar to that of *Gleichenia* in appearance. No hairs are borne on the gametophyte.

3. The antheridium is of a primitive type with a wedge-shaped basal cell and a wall consisting of four to six additional cells. The cap cell divides once or twice, forming an opercular cell which is thrown off at maturity. The antheridium varies greatly in size, and the number of sperms may be considerable. One diameter of the antheridium may show five to eleven sperms.

4. The archegonium develops in the usual manner of the leptosporangiate ferns. The neck is straight and unusually

long; it is directed forward on the younger parts of the gametophyte, but on the older parts it is directed laterally or downward.

5. Apogamy is of frequent occurrence.

6. The evidence given by the gametophyte of *D. conjugata* suggests a closer relationship to the more primitive ferns, particularly the Gleicheniaceae, than to the Polypodiaceae, and indicates that the family Dipteridaceae established on the basis of sporophytic characters alone is also justified by the structure and development of the gametophyte.

The writer wishes to express her thanks to the members of the staff of the Botanical Gardens at Buitenzorg and Tjibodas, Java, for their generous assistance in facilitating the collection and culture of the material used in this investigation.

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EXPRESSION OF CERTAIN HEREDITARY FACTORS IN YELLOW BERMUDA ONIONS INDUCED BY UNSEASONABLE PLANTING IN THE GREENHOUSE

S. S. IVANOFF

Introduction

Of the commonly grown crop plants, the onion (*Allium cepa* L.) seems to be unusually sensitive to certain changes in the environment. It is a common experience among growers in southern Texas to have a crop of Yellow Bermuda onions lacking uniformity, particularly in amount of bulbing, size, maturity, doubling and splitting of bulbs, and number of seed stems produced. Bulbing in onions is definitely determined by the photoperiod during the growing season, and under southern Texas growing conditions only a few varieties are able to produce good commercial bulbs (1). Undoubtedly some of the variations are basically due to lack of genetically pure varieties adapted to local conditions. HAWTHORN (2) has shown that inbreeding, combined with selection away from undesirable characters of doubling and

premature seeding, may result in lines of onions free from these characteristics. Recently the same writer (3) has developed through selection from the heterozygous Early Grano onion a new strain, the Texas Grano, which shows a remarkable degree of uniformity as to size, bulbing, and early maturity. Such variations, however, may also be attributed to the influence of local environmental conditions under which the crop is grown and which may remain expressionless under a different environment. For example, the number of doubles and splits—and particularly bolters[1] may vary significantly from season to season, farm to farm, place to place on the same field, and even within the same section of row. Such variations, which depend principally on environment, have been observed in both open-pollinated varieties and inbred lines of Bermuda onions.

This report deals with outstanding variations as manifested by different plant forms and behavior within a population of onions which were induced by unseasonable planting of seed in a greenhouse, where the plants sometimes grew at unusually high temperatures and light intensities. The results were obtained in an attempt to shorten the life cycle of the plant from 2 years to 1 year by various means, as a practical aid in hastening the work on breeding for disease resistance. (Under ordinary conditions, the bulb crop in southern Texas is harvested in April or May. To obtain seed from the bulbs, the latter need to pass through a rest period in storage during the summer, after which they are planted in November. Seed from these bulbs is obtained in May or June of the following year. Under these conditions of culture, the onion plant completes its life cycle in about 22 months.)

[1] Plants which form seed stalks prematurely.

Differences in bulbing within a population in response to definite sets of controlled environmental conditions in the onion have been recorded by other workers. McCLELLAND (5) studied different onion varieties in their response to different photoperiods and found that, while one variety may bulb 100% under a certain photoperiod, in another variety only certain individual plants may bulb under the same photoperiod. MAGRUDER and ALLARD (4) and WILSON (9) recorded similar results. MAGRUDER and ALLARD worked with eighteen varieties grown under various photoperiods and found that Yellow Bermuda was the only variety that produced normal bulbs under the 10-hour light treatment, but only 10% of the plants within the population were able to do so. With longer photoperiods other varieties performed similarly, as some plants within the group formed good bulbs while the rest of the plants failed to bulb. WILSON, studying the influence of hydrogen-ion concentration on growth of onions, found that within a given reaction series bulbing occurred first on the least thrifty plants. The work of ROBERTS and STRUCKMEYER (6), because of its emphasis on variations within the same population of plants, has closer relation to the studies here reported. They investigated the influence of temperature and light periods on some forty-three species and varieties and found that seedlings, and in some cases both seedlings and clones, may have either uniform or variable populations—depending upon the environment in which they were grown. For example, plants of pennycress (*Thlaspi arvense*) grown from seed appeared more uniform in size and other characteristics in a cool, long-day environment than in a cool, short-day environment. On the other hand, clones

of *Rudbeckia laciniata* were more variable in cool than in warm photoperiods. However, since they found that clonal populations were also subject to similar variations as the seedling populations under certain conditions, it is not likely that these variations were due in all cases to genetic diversities. It must be concluded that other factors influenced variability. The same workers (7) later reported that Chinese cabbage showed a relatively uniform type of growth in cool temperatures, particularly on long photoperiods, but showed quite variable growth in warm temperatures, particularly on short photoperiods. They collected seed (open-pollinated) from the different types of plants from the same population. The seedlings from these types, however, did not show the differences of the parents when grown in a cool location, but they did tend to be like the parent plants when grown in a warm location. Recently YARNELL (10) has summarized the work pertaining to the influence of environment on the expression of hereditary factors as related to problems of breeding in southern United States.

Procedure

Two plantings of onions grown from the same lot of seed were compared. One lot was grown during the usual period for onion production in the Winter Garden region and the other was raised during the summer in the greenhouse. For the seasonable crop, seed was planted in an open seedbed in early fall, the seedlings transplanted in late November or December, and the mature bulbs harvested the following April. With the unseasonable plants the seed was planted in June in a greenhouse where the temperatures often reached 125° F. or higher and were 4°–35° higher than outside. The greatest differences in temperature between the two crops occurred during the summer months when the greenhouse plants were in their seedling stage, and again in the following spring when the bulbs were maturing. In the case of the seasonably planted onions the highest average temperature occurred as the bulbs reached maturity.

There were two experiments of this kind, each 1 year in duration. The second experiment was conducted 1 year after the first and was intended to be essentially a repetition of the first. In the first experiment some of the unseasonable plants were transplanted into jars of soil or to the field to avoid crowding. A third experiment, conducted from October, 1943, to April, 1944, was designed to test the behavior of the second-generation plant progenies derived from some of the plant material grown in the first trial.

Seed of the Yellow Bermuda variety was used: one lot of a first generation inbred line and a second lot of an open-pollinated commercial stock. The photoperiods during the summer and winter solstices at Winter Haven, Texas, where the trials were made, are approximately 14.1 and 10.2 hours, respectively.

Experimental results

UNSEASONABLE PLANTS

Seed of Yellow Bermuda (first-generation inbred) onions was planted on June 1, 1942, in a greenhouse bench in uniform, light sandy loam. Abundant space was provided to avoid crowding of the young plants. There were forty plants in all. Following emergence, the plants grew normally for several weeks, after which some of them began to bulb and finally formed sets,[2] at which time the

[2] Onion sets are merely undersized (usually not more than $\frac{3}{4}$ inch in diameter) mature onion bulbs raised from seed and arrested in growth. Sets re-

leaves withered. The sets matured at different times (about 55–120 days after planting the seed) and varied from 8 to 37 mm. in diameter. There were twenty-two sets formed, or 55% of the total number of plants. The sets were harvested as they matured, and after passing through a rest period for 6–8 weeks they were planted in the fall and the winter; some were planted in the field and others in jars of soil kept in the greenhouse. All these sets developed into single-bulb plants in April and May of the following year, without seed stalks or seed.

The other plants which did not form sets (referred to hereafter as non-set-formers) appeared as normal young green plants with erect turgid tops and no conspicuous swelling at the base. After some time, however, certain significant variations in form were distinguished among them. Thus, on December 1, 183 days after planting, in addition to the sets already mentioned, there were two other distinct forms: plants which had not yet started to bulb and plants which had formed large bulbs, reaching their ultimate size of $3\frac{1}{2}$–4 inches or more in diameter (fig. 1*A*–*C*). Several months later, at harvest in the following spring and summer when all plants had reached maturity, there were altogether three distinct plant types: (1) 25% were large single bulbs which matured without producing seed stalks; (2) 50% were large multiple bulbs (two to five per plant) that had matured without producing seed stalks; and (3) 25% were multiple bulbs with seed stalks bearing large normal-sized umbels and viable seeds. All the plants matured in less than a year after planting the seed, and some of them (type 3) completed their entire life cycle from seed to seed within that time. A diagrammatic presentation of the various onion forms produced at different times is shown in figure 2.

sume growth when planted following a short rest period. Ordinarily they are produced in the northern states by sowing the seed thickly so that the bulbs reach their maximum growth quickly, usually within 3–4 months after planting the seed (8). Sets have been produced in southern Texas in similar manner.

Seasonable plants

Following the usual practice of raising onions in this region, seed was planted in an outdoor seedbed on August 28, 1942, the plants were moved to the field on November 24, and the bulbs harvested in late April of the following year. These were considered as control plants. Two kinds were used: 201 plants raised from the same inbred lot of seed as was used in the greenhouse planting, and 477 plants raised from open-pollinated commercial Yellow Bermuda seed. The latter were included in this trial for the purpose of comparing the behavior of inbred-line onions with that of an open-pollinated variety. At the end of the season, 98% of the inbred-line onions were single bulbs, 1% were bolters, and 1% were doubles; of the open-pollinated onions, 94% were single bulbs, 1% were bolters, and 5% were doubles. The difference in behavior of the two kinds of control plants was relatively slight, but the difference between both kinds and the greenhouse plants was striking. The differences were evident mainly in the type of plant forms produced. The field-planted seed did not produce sets, and none of the plants developed into multiple-bulb, seed-producing types—as was the case with the greenhouse plants. On the other hand, 1% of the control plants were bolters, and none of this type occurred among the greenhouse plants.

There are significant differences in appearance between the bolters found in

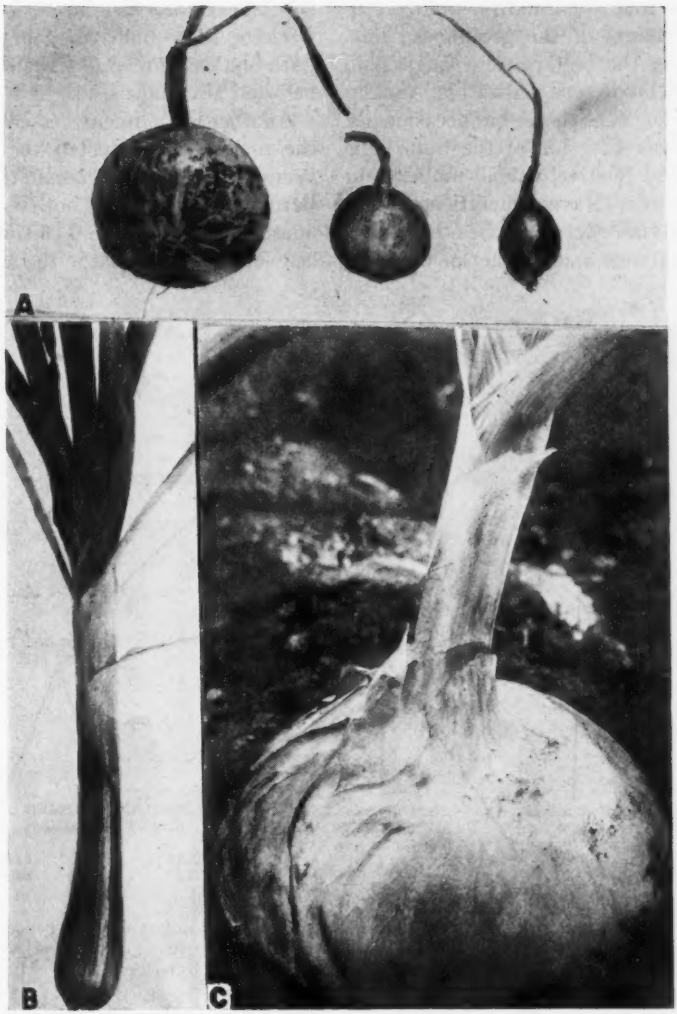

FIG. 1.—Three variations in Yellow Bermuda onions as they appeared on December 1, 1942, 183 days after planting the seed unseasonably in greenhouse: *A*, sets of various sizes; *B*, bulbless plant; *C*, large bulb form nearing its ultimate size. Several months later, variations shown at *A* developed into large single bulbs; variations at *B* developed into three types (single bulbs, multiple bulbs without stems, and multiple bulbs with several seed stems bearing viable seeds); and variations at *C* developed into multiple bulbs without stems or multiple bulbs with seed stems bearing viable seeds. Twenty-five per cent of the non-set-formers (*B* and *C*) completed their life cycle in less than a year. $\frac{3}{4}$ natural size.

the field and the multiple-bulb, seed-bearing plants of the greenhouse lots. As a rule, the bolters were single-bulb plants with one seed stalk which usually arose through the center but occasionally occurred on the side of the bulb. The bolters had relatively small umbels and under Winter Haven conditions rarely produced viable seed. On the other hand, the appearance and manner of development of the unseasonable multiple-bulb, seed-producing plants which completed their life cycle in less than 1 year were indistinguishable from those of the commercially grown seed-producing plants at the end of their second-year period. With these 2-year-cycle plants, which are raised from large bulbs grown the season before, the "mother-bulb" before disintegrating produces several or more shoots, each of which later forms a bulb. Each of these bulbs may in time produce one or more stalks with large umbels and abundant seed.

Another experiment, essentially like the first, was conducted the following year. However, open-pollinated Yellow Bermuda seed was used both in the greenhouse and in the field. The soil was of a heavier type than that in the first experiment. Seed was planted in the greenhouse on June 20, 1943. Ample space was again provided for the emerging plants in order to avoid conditions favorable for set formation. All seeds were planted at a uniform depth of ½ inch. Of the thirty-two plants obtained, 53% formed small sets by the middle of September, after which no more sets were formed. The rest of the plants developed as stout

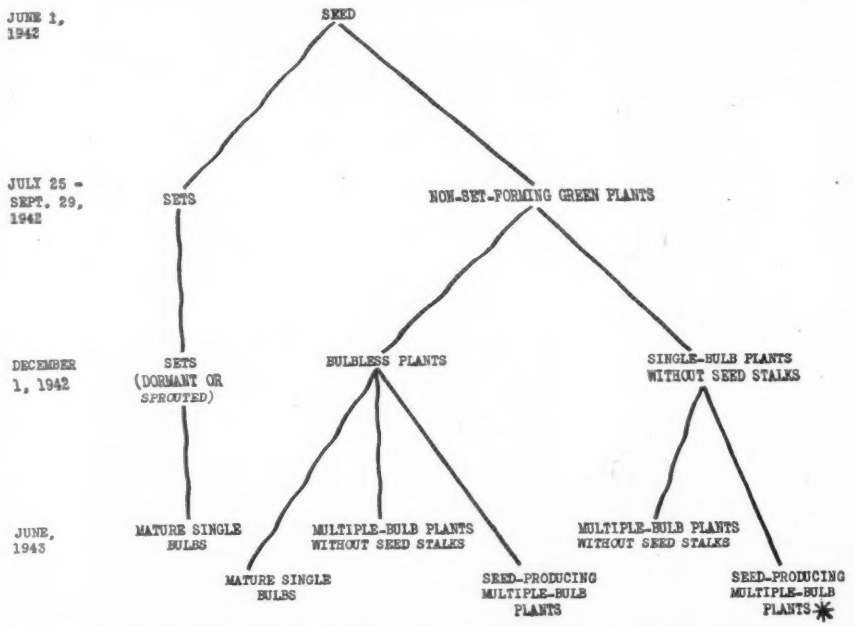

FIG. 2.—Diagrammatic presentation of various onion forms produced at different periods from seed planted unseasonably in greenhouse (experiment 1). (*Seed from one plant of this type, when planted seasonably in the field, produced 92% single bulbs, 8% doubles, and no bolters.)

seedlings and continued to grow without bulbing for some time. These plants were left in place in the greenhouse bench until the experiment was completed, and they finally developed into multiple-bulb forms, all but two of which (87%) produced viable seed in May, 1944. Like the plants of the first experiment, they also completed their life cycle in less than a year. After a rest period the sets were gle-bulb bolters, and 3% doubles. There were no multiple bulbs produced in the outdoor planting. The results of the two experiments are summarized in table 1.

A third experiment was conducted to determine what kind of plants would develop from the seed produced by the plants grown in the greenhouse which completed their life cycle in 1 year (type 3), when the seed is planted in season in

TABLE 1

TYPES OF YELLOW BERMUDA ONION PLANTS PRODUCED BY UNSEASONABLE PLANTING AND SUMMER CONDITIONS IN THE GREENHOUSE, CONTRASTED WITH FIELD PLANTING IN SEASON, AT WINTER HAVEN, TEXAS

Strain or variety	Location and date of seed planting	Total no. of plants	Sets produced (%)	Type developed from sets (%)			Type developed from non-set-formers (%)		
				Single bulb	Multiple bulb without stems	Multiple bulb seed-producers	Single bulb	Multiple bulb without stems	Multiple bulb seed-producers
Inbred	Greenhouse June 1, 1942	40	55	100	0	0	25	50	25
Inbred	Field Aug. 28, 1942	201	0	.....	.....	.....	99*	1‡	0
Open-pollinated commercial	Field Aug. 28, 1942	477	0	.....	.....	.....	95*	5‡	0
Open-pollinated commercial	Greenhouse June 20, 1943	32	53	100	0	0	0	13	87
Open-pollinated commercial	Field Oct. 5, 1943	532	0	.....	.....	.....	97†	3‡	0

* 1% single-bulb bolters. † Including 0.3% single-bulb bolters. ‡ All were doubles (two-bulb plants) and splits.

planted again, some in the greenhouse and others in the field. All of them developed to maturity as single bulbs, as was the case with the sets in the first experiment.

For controls in this experiment, there were 532 plants raised from seed planted in an outdoor seedbed on October 5, 1943, and transplanted to the field on December 24. In the following April, when the bulbs were harvested, there were about 97% single bulbs, 0.3% single bulbs, and 2.7% doubles. There were no multiple bulbs produced in the field. It was thought that seed raised in this way might produce plants with a tendency to bolt.

Seed from one of these plants of the first experiment was planted in the seedbed October 5, 1943, a few months after it was harvested. The plants were transplanted to the field on December 22 and harvested when the bulbs matured (during the latter part of April, 1944). There were 110 plants in all. For checks there were 206 plants of open-pollinated com-

mercial Yellow Bermuda which were raised at the same time and handled in the same manner as the first lot. The check plants were grown in two rows, one on each side of the row where the above-mentioned lot of plants were grown. The results at harvest showed no bolters among the progenies of the type-3 plant and only 0.4% of bolters in the check plants. The low percentage of bolting in the commercial open-pollinated Bermuda is an indication that the environmental conditions were not very favorable for bolting. On the other hand, there were 8% of doubles in the type-3 progenies and 3% in the checks. These results show no significant difference in regard to amount of bolting between the two lots of plants. Also, the progenies of the type-3 plants were not particularly inclined to bolt under the conditions of the experiment.

Discussion

The results reported pertain to the influence of an unusual environment on the hereditary behavior of the Yellow Bermuda onion. The high temperatures, long photoperiods, and high light intensities in the greenhouse during the summer are associated with the development of various plant forms which are seldom if ever encountered in seasonably grown onions in the field. Some seedling plants interrupted their development by forming sets; others continued their growth without interruption and developed at different times into various forms—such as single bulbs, multiple bulbs without seed stalks, and multiple bulbs which produced seed. Some of these forms were entirely absent in comparable lots grown under lower temperatures, short photoperiods, and lower light intensities of the normal onion-growing season.

In interpreting these results, and in similar studies by other workers, the degree of genetic diversity of the plants employed should perhaps be considered. The inbred line of onion seed may be assumed to be genetically less heterozygous than the open-pollinated lot, but, in view of the results obtained, its uniformity apparently was not of a high degree. On the other hand, the open-pollinated seed stock may be expected to be genetically more variable and is likely to give rise to a number of different genotypic lines, as the onion plant is partly self-pollinated and partly cross-pollinated. As a consequence, within a lot of commercial open-pollinated seed there may be individual seeds which are the result of hybridization between two plants and others which are of inbred nature. Some of the lines arising from such seed will be first-generation inbreds, while others, progressively fewer in number, may be second-, third-, or even later-generation inbreds. Since onions lose vigor by inbreeding, and since vigor—according to observations made by this writer—seems to be associated with a tendency for doubling and bolting under field conditions, it may be possible that the factors for hybrid vigor are partly responsible for the development of some of the variations in these trials. Perhaps in all similar studies the factor of the degree of cross-pollination of the experimental plant species should be taken into consideration.

Some applications of these results to onion breeding seem evident. The shortening of the life cycle of the onion from 2 to 1 year would be of great help to the breeder, provided the plants which complete their life cycle in 1 year are not segregates for the bolting habit in the field. It is possible that under certain conditions of culture perhaps all the in-

dividuals of a certain population may shorten their cycle to 1 year, as the results of the second experiment indicate. In this experiment 87% of all the non-set-formers completed their life cycle in less than a year, as compared with only 25% of the same group of plants in the first trial. These differences in results between the two trials may be accounted for in part by the differences in environment of the two seasons.

Even commercial application of these results may be suggested, in that seed may be produced in one growing season in certain areas by planting early and directly in the field. Such practice would considerably reduce the cost of seed production. Recently a seed-to-seed crop of Yellow Bermuda in 1 year was produced unintentionally by growers near El Paso, Texas, by direct planting of seed in September. Under the favorable conditions provided approximately 90% of the plants went to seed, but the great majority of the "seeders" had only one seed stalk and the yield of seed was light. By further experimentation, such as planting the seed more thinly or thinning out the plants early in the season, it may be possible to induce all or nearly all the plants to seed in 1 year and to produce several seed stalks. Recently, such attempts have been made by commercial seedsmen elsewhere. However, a word of caution in regard to such practice is necessary. Since not all plants may go to seed, it is likely that in the course of several generations the genetic composition of the onion variety may be altered to such an extent—particularly in regard to bolting—as to make it undesirable for raising bulb crops. It would be necessary in such cases to raise and maintain a foundation seed stock of the original variety. Certain varieties other than Bermuda, such as Early Grano, however, are known to bolt either not at all or only negligibly under seasonable conditions in some regions. With these varieties the chances of changing to a bolting type would be correspondingly less than in case of the Bermuda onion.

The studies on the influence of environment on the expression of hereditary factors, as far as the practical breeder is concerned, point to the conclusion that the breeding of superior varieties of plants should be done in the region where the variety is intended to be grown commercially. The aim of the plant breeder should be to bring out and fix those genetic characters of a plant which would best enhance its economic value as a commercial crop in a particular locality.

Summary

1. Studies are reported from two trials on outstanding variations in form and behavior among individuals within a population of Yellow Bermuda onion plants, brought about by unseasonable planting (in June) of the seed in the greenhouse, where the plants sometimes grew at unusually high temperatures and light intensities. These developments were contrasted with the lack of similar variations in seasonably grown plants (from fall through spring) in the field.

2. The unseasonably grown plants produced within a year (*a*) onion sets, all of which, planted after a rest period, developed into single large bulbs; (*b*) single bulbs of various sizes; (*c*) multiple bulbs (two to five per plant) maturing without producing seed stalks; and (*d*) multiple bulbs producing seed stalks and viable seed. Seed from the last group of plants, when planted in season, produced mostly single bulbs with no seed stems.

3. From 10 to 40% of all unseasonably

grown plants completed their life cycle in less than a year, forming multiple bulbs and stems with viable seed.

4. The seasonably grown plants in the field produced mostly single bulbs and a few doubles, splits, and bolters.

5. Some practical applications of the results of these studies are suggested.

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EFFECT OF INDOLEACETIC ACID IN INHIBITING STEM ABSCISSION IN MIRABILIS JALAPA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 566

J. M. BEAL AND A. GERALDINE WHITING

Introduction

Several investigators have reported on the effectiveness of certain of the growth-regulating substances in delaying abscission of plant organs (**2**, **3**, **5**, **8**, **9**), an effect of considerable economic importance to many plant growers. A study of the anatomical effect of some of these substances on abscission of the leaves of *Coleus* has been reported by MYERS (**8**). The abscission of a stem or fruit, however, may differ structurally from the process which occurs in the leaf petiole, and it is possible that these differences may affect the response to the growth substance. The influence of indoleacetic acid on stem abscission in *Mirabilis jalapa* has been briefly mentioned by HAMNER (**4**). Further observations on the effect of indoleacetic acid in inhibiting stem abscission in that plant are reported herewith.

METHODS.—The plants used in this investigation were grown during the summer of 1939. Seeds of four o'clock, *Mirabilis jalapa* Linn., were planted in good garden soil in pots and the resulting seed-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

lings kept on an evenly lighted greenhouse bench, where they received the care necessary to insure good growth.

Three groups, selected for uniformity of size, were chosen from these plants for the studies. The plants of one lot (group 1) were permitted to grow without additional treatment and constitute the controls. Those of group 2 were decapitated by cutting squarely across the first internode when the second internode had just started to elongate. The length of first internode remaining after decapitation averaged about 0.5 inch. Plants of group 3 were decapitated in the same manner, but immediately after decapitation the cut surface of the internodes was covered with a 2% indoleacetic acid-lanolin mixture. The three groups were subsequently kept under as nearly identical growing conditions as possible.

Material for histological study, consisting of the cotyledonary node with adjacent portions of the hypocotyl below and of the first internode above, was collected at various intervals, fixed in Navashin's solution, and carried into paraffin through the tertiary butyl-alcohol method. Sections were cut at 10μ and stained in a modified triple stain.

Observations

GROSS RESPONSES.—The untreated plants of group 1 showed continued development of the main axis from the terminal buds. The axillary buds did not expand noticeably, and there was no indication of abscission of stems in any of these plants during the time of the experiments.

The plants of group 2, which were decapitated and not further treated, responded by marked extension and development of the axillary buds at the cotyledonary node. Generally there were present also accessory buds which developed at almost the same rate as the axillary ones. Although the decapitated first internodes made no further appreciable growth, they frequently remained green for as long as 2 weeks after decapitation, and then they usually became yellowed and dropped from the plants while still relatively turgid or after becoming much shriveled. Practically all of them had fallen at 3 weeks.

The plants of group 3 which were decapitated and treated with the indoleacetic acid-lanolin mixture behaved in essentially the same manner as has been described by HAMNER. While the growth of buds in the axils of the cotyledons is greatly delayed, they nevertheless grow rapidly after about the sixth day following application of the lanolin mixture. These internodes develop apical tumors and, according to HAMNER, may remain firmly attached for several months.

HISTOLOGICAL RESPONSES.—Longisections of the cotyledonary node of untreated controls showed that growth in diameter continued in these stems (figs. 1*A*, 2*A*, *B*). The small tiered cells at the base of the internodes suggest that elongation is continued in this region. The axillary buds remained unexpanded and essentially unchanged. Abundant starch was stored, chiefly in the pith (fig. 2*A*, *B*). No indications of the development of an abscission layer were observed in any of these sections.

The plants which were decapitated and allowed to develop with no additional treatment behaved in the same manner as has been noted by both LLOYD (**6**) and HAMNER. There is neither increase in diameter of the internodes following decapitation nor additional secondary thickening. As early as 3 days after decapitation these internodes showed shrunken and collapsed cells in the pith, resulting in the formation of cavities (fig.

1*B*). Active growth had already begun in the axillary buds at this time, but cells of the hypocotyl and of the cotyledonary node seemed unaffected.

upper one extends entirely across the base of the internode while the lower extends outward only as far as the outer ring of vascular bundles. In sections cut

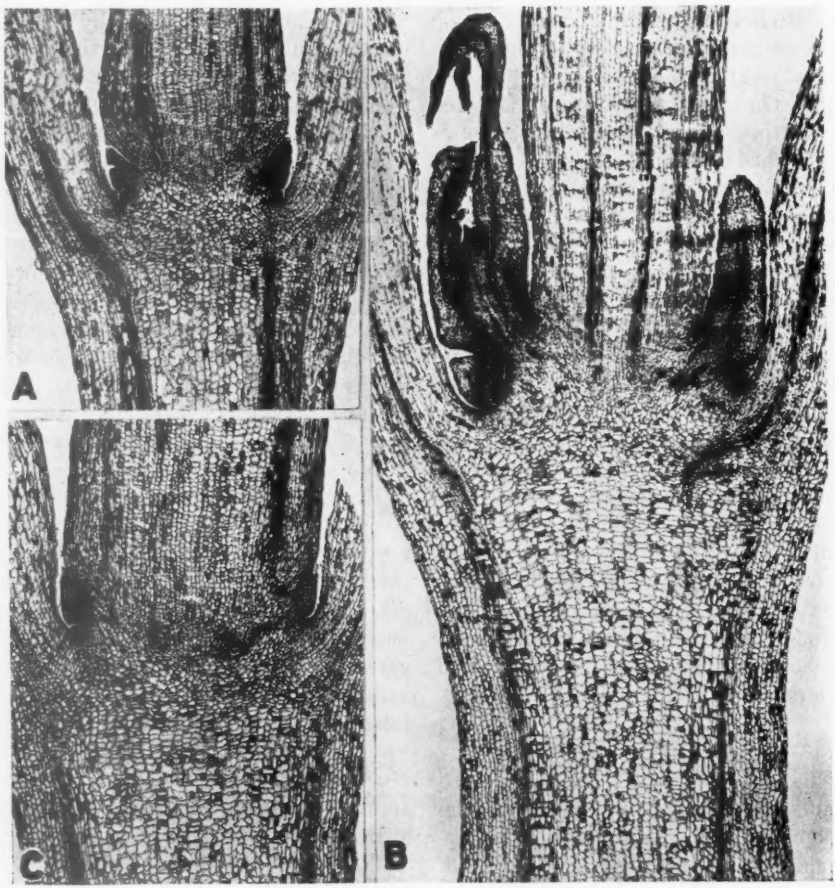

FIG. 1.—Longisections through cotyledonary node: *A*, control plant showing axillary buds and general turgid condition throughout; 8 days after beginning experiment. *B*, decapitated plant without indoleacetic acid, showing cavities in internode resulting from shrinkage and collapse of cells, and developing axillary shoots; 3 days after decapitation. *C*, plant decapitated and treated with indoleacetic acid, showing lateral buds inactive, internode larger in diameter, and cells generally turgid; 3 days after decapitation and treatment.

The earliest indication of an abscission layer was found at 14 days following decapitation (fig. 3*A*, *B*). Here two meristematic regions are clearly apparent; the in the cotyledonary plane this zone connects with the vascular bundles of the axillary shoots, while those cut in the intercotyledonary plane show connection

with the outer bundles ascending from the hypocotyl. While the lower meristem has been observed in a number of stems in which no indication of the upper could be detected, there is no evidence pointing to its direct participation in abscission. Whenever the upper of these two regions has been found, the lower has been present without exception. It clearly is not a part of the actual abscission layer, however, but just what its relation may be to abscission or other associated activities is not known.

Meanwhile, the axillary buds, and usually also the accessory buds, have been developing rapidly (fig. 4*A*, *B*). The diameter of the decapitated internodes remained relatively unchanged. Many of the internodes had shed by the eighteenth day following decapitation, but others remained for more than 24 days—at which time the experiments were terminated. In none of these plants was it possible to demonstrate storage starch, as was possible in the controls.

An interesting, and perhaps the characteristic, condition immediately preceding abscission is shown in figure 7. The internode, although apparently not materially shrunken, had not increased in diameter. Collapse and disintegration of cells, especially in the pith, resulted in the formation of conspicuous cavities. Continued growth in diameter of the hypocotyl and cotyledonary node had set up considerable differential tension between the latter region and the base of the internode. When an abscission zone developed, the break appeared to begin at the surface region of the internode as a result of the differential tension. Many of the cells at the base of the internode became crushed and appeared as a black line extending across the stem (fig. 7*A*, *B*). On the slides these cells stain intensely with orange G. When the internode abscised, these crushed cells appeared to remain attached and were carried away with it, since none remained on the stems examined (fig. 6). Subtending the crushed cells is a zone of suberized cells, and it is at the upper margin of this zone that the actual break appears to occur in the process of abscission in *Mirabilis*. The line along which the break occurs has been called the "separation layer" by LLOYD, who reported it to consist of one to five tiers of cells in older organs, such as internodes. Our material shows that it comprises at least five tiers of cells, possibly more (figs. 6, 7), for the line of separation is highly irregular.

The lower or proximal portion of the abscission zone consists of meristematic cells which proliferate freely during and following abscission (figs. 6, 7). This region functions as a phellogen, although it comprises a number of layers or tiers of cells.

Lying across the stelar portion of the stem, between the abscission zone and the lower meristem, is a zone of parenchymatous cells which apparently remain relatively unchanged during all this time. While divisions, associated with the growth in the cotyledonary zone, occur in some of these cells, the group as a whole appears to play no active role in the process of abscission.

Plants which were decapitated and treated with the indoleacetic acid-lanolin mixture (fig. 1*C*) showed certain similarities to the controls. As noted earlier, the internodes increased in diameter and showed essentially no shrinkage or collapse of cells in the pith or other regions. And while the buds in the axils of the cotyledons were inhibited or definitely delayed in development, they generally grew rapidly, beginning about the sixth day following treatment (fig. 5*A*). None of these plants showed evidences of an

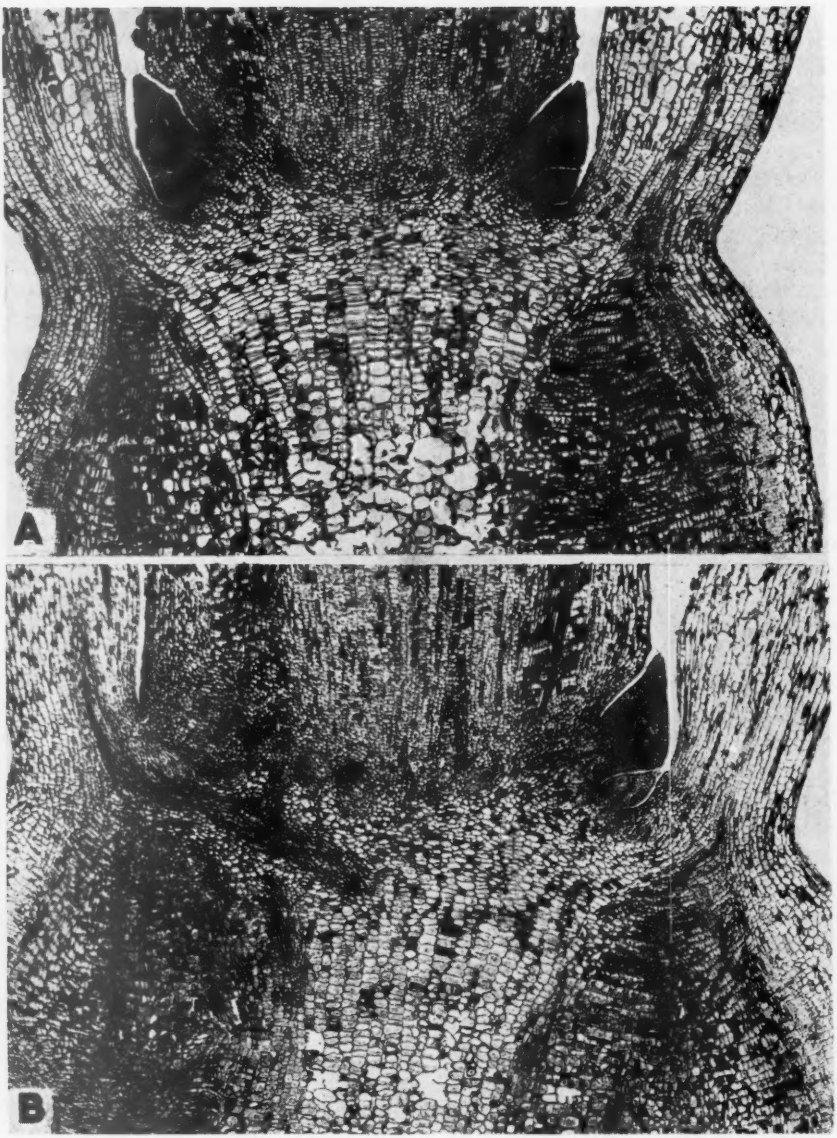

FIG. 2.—*A*, longisection of control; 18 days after start of experiment. *B*, same at 24 days; axillary buds still inactive, cells throughout sections generally turgid, those of pith and rays containing starch.

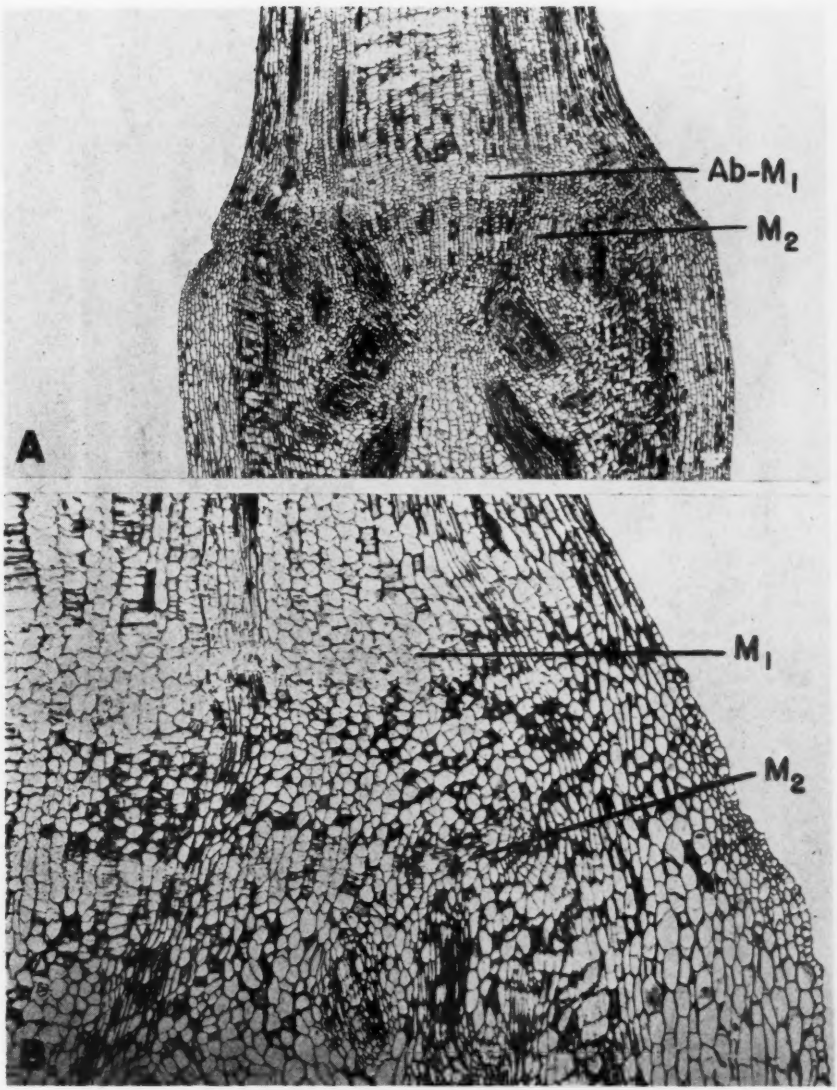

FIG. 3.—*A*, longisection of decapitated plant through intercotyledonary plane, showing developing upper meristematic region or abscission zone (Ab–M_1) and lower meristematic zone (M_2); *B*, same at higher magnification to show details. Fourteen days after decapitation.

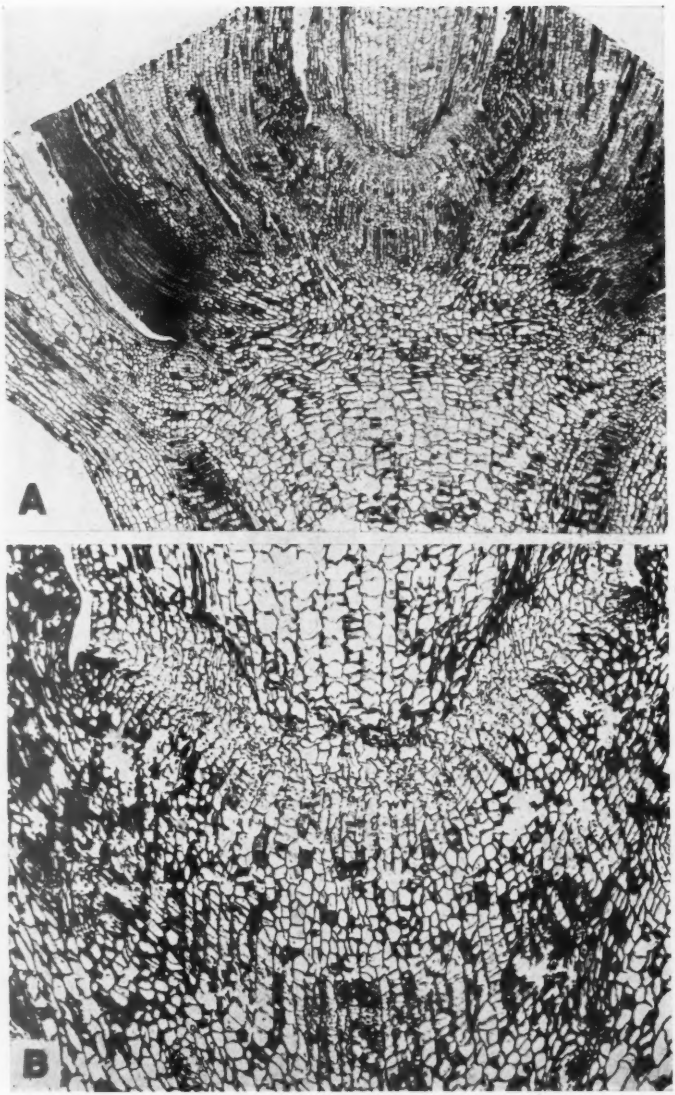

FIG. 4.—*A*, longisection of decapitated plant with well-developed abscission layer and lower meristematic zone; pith shows shrunken cells and cavities; axillary shoots have greater diameter than internode; *B*, same section showing details of abscission and lower meristematic zones. Eighteen days after decapitation.

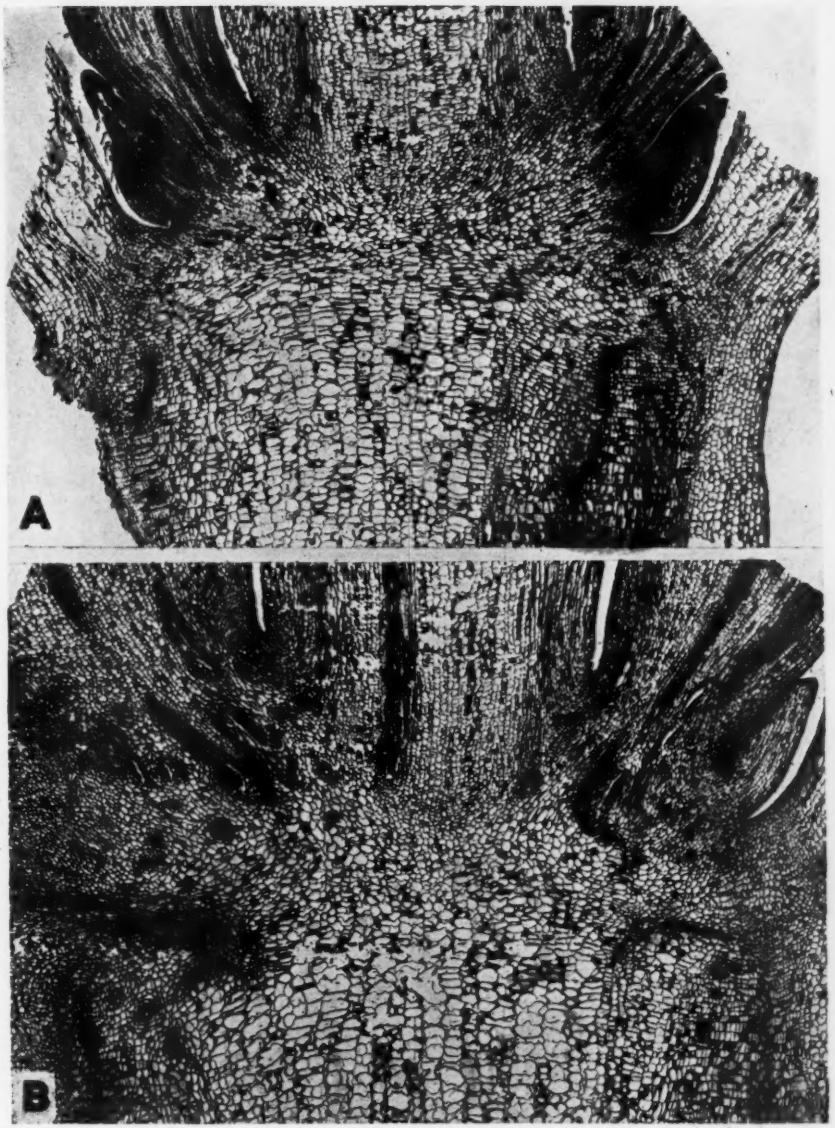

FIG. 5.—*A*, longisection of plant decapitated and treated with indoleacetic acid at 14 days. *B*, from plant treated in same way at 24 days following treatment; cells generally turgid, axillary shoots prominent, and internodes increased in diameter; no indication of abscission zone.

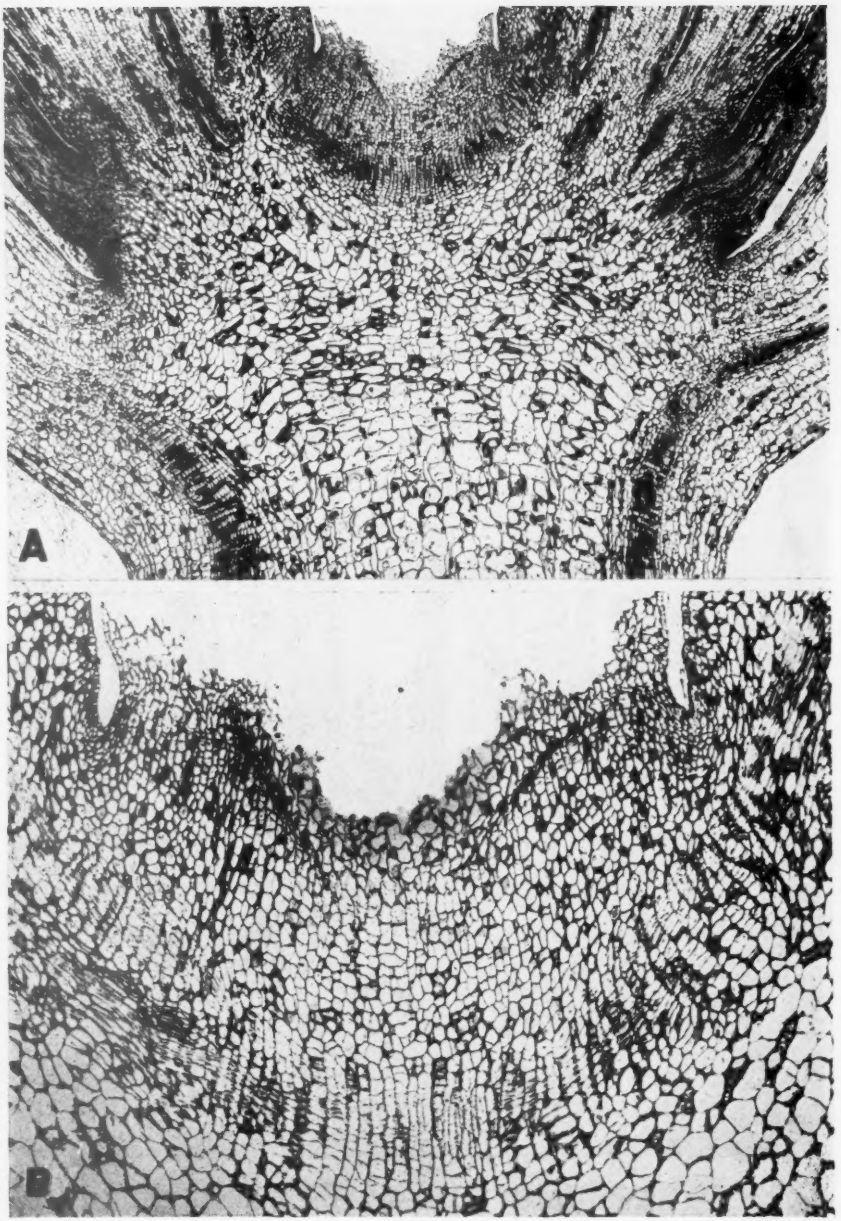

FIG. 6.—*A*, longisection of decapitated plant through abscission zone from which internode has abscised and also second meristematic zone below; *B*, details of the two zones from *A*; outer two to five layers of cells appeared to be suberized. Eighteen days after decapitation.

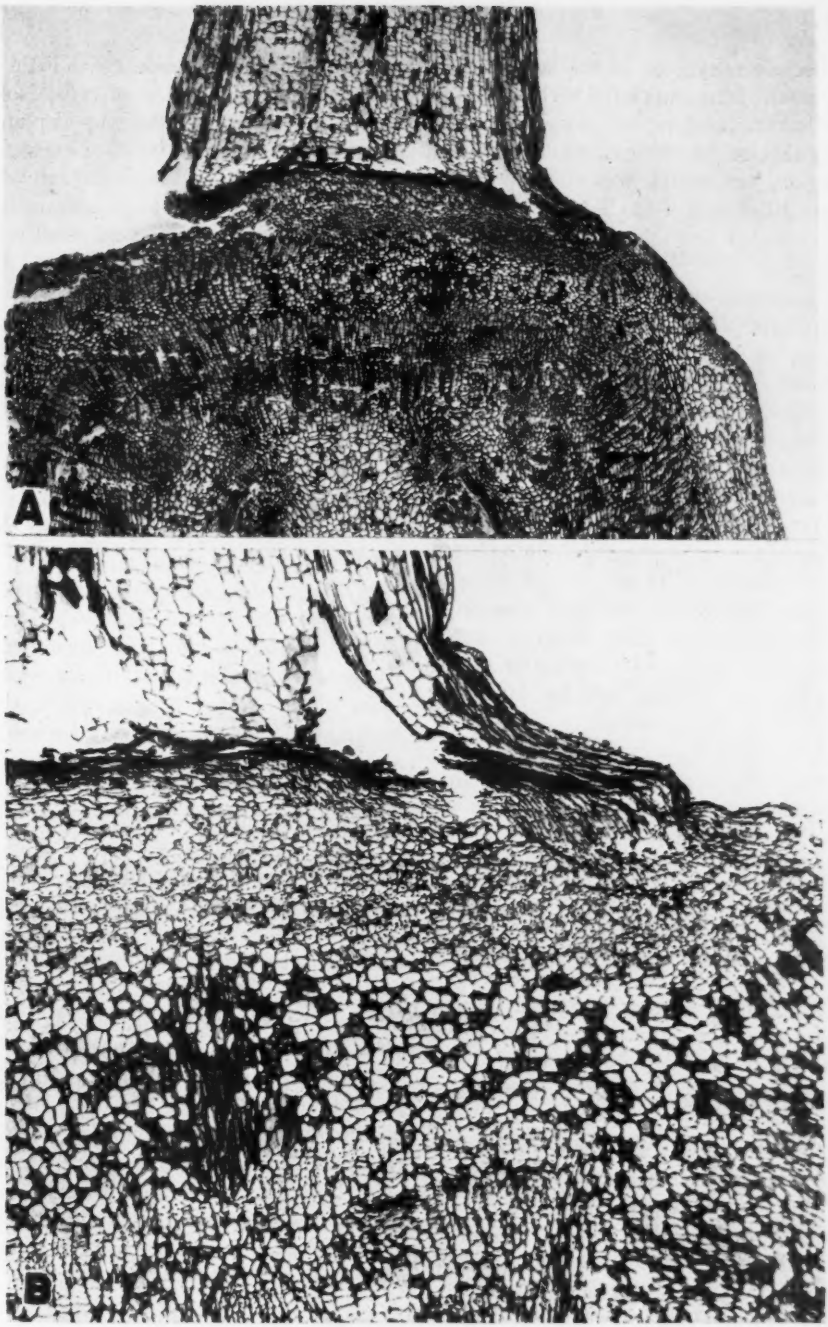

FIG. 7.—*A*, longisection through intercotyledonary plane of decapitated plant, showing abscission and lower meristematic zones; *B*, details of right side of *A;* black streak at base of internode consists of crushed internode cells; abscission occurs below these cells. Twenty-four days after decapitation.

abscission layer or of the second meristematic zone associated with it. The indoleacetic acid incited no visible histological changes in the cotyledonary nodal region, and starch was stored in small quantities only (fig. 5*B*).

Discussion

A comparison of the two groups of decapitated plants with the untreated controls, in which abundant starch was stored, indicates that the physiological conditions have been greatly disturbed by the decapitation, whether indoleacetic acid is applied or not. The disappearance or absence of starch from the decapitated plants to which indoleacetic acid was applied is similar to the condition reported by BORTHWICK, HAMNER, and PARKER (1) for the tomato following treatment with indoleacetic acid. That an actual hydrolysis of stored starch follows the application of this and similar acids has been demonstrated by MITCHELL, KRAUS, and WHITEHEAD (7), as well as by other investigators since. Those plants which were decapitated and given no additional treatment failed to store much starch during the period of the experiments. Very small amounts of starch were observed, however, in the pith, ray, and inner cortical cells of these stems.

Not only is there an effect upon the quantity of stored starch, but the growth pattern is markedly altered through a redirection of growth from the main axis into the axillary shoots. There is also a growth difference between the two groups of decapitated plants. Those given no further treatment showed practically no additional growth in the internodes, which usually abscise within 3 weeks, although some remain attached longer. On the other hand, those decapitated and treated with indoleacetic acid showed an appreciable increase in diameter of the internodes, with little or no shrinkage or collapse of cells. None of the internodes of this group of plants abscised during the period of 24 days, nor were evidences of an abscission zone detected in any of the longisections observed. The indoleacetic acid effectively inhibited abscission of the petioles of *M. jalapa* for the period of 24 days during which these observations were made.

Summary

1. Untreated, intact plants of *Mirabilis jalapa* showed vigorous growth of the main axis with neither abscission nor the formation of an abscission zone in internodes during the period of these experiments.

2. Plants decapitated in the upper portion of the first internode when the second internode had just started to elongate and given no additional treatment showed cessation of growth in the internodes, followed by their abscission after 2 weeks or longer. Preceding abscission, two transverse meristems developed. The upper formed across the base of the internode and developed into the zone in which abscission occurred. The lower meristem, invariably present when the upper developed, seemed to play no active role in the process of abscission. Relatively little storage starch remained in the cotyledonary nodal region of these plants at 2 weeks after decapitation.

3. Plants similarly decapitated and treated with a 2% indoleacetic acid-lanolin mixture on the cut surface showed continued growth of the internodes, complete disappearance of stored starch, and the entire absence of abscission or of an abscission zone at the bases of the internodes.

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MORPHOLOGICAL CHANGES IN PEACH SEEDLINGS FOLLOWING AFTER-RIPENING TREATMENTS OF THE SEEDS[1]

H. B. TUKEY AND R. F. CARLSON

Introduction

Peach seeds (*Prunus persica* Batsch) will not germinate immediately after they reach maturity; they must first be conditioned or after-ripened. The after-ripening processes proceed most rapidly at 3°–5° C. in a moist medium, such as damp sand or peat moss. Ten to twelve weeks have been found adequate for after-ripening the types of peach seeds used by the nursery trade and in commercial horticultural enterprises.

When non-after-ripened seeds are dissected, so that the endosperm, nucellus, and integuments may be removed and the embryos placed in an environment favorable to germination, they will germinate and grow without previous after-ripening. The seedlings which develop, however, show dwarfed and anomalous growth characteristics (1, 4). The leaves are sometimes short and broad rather than lanceolate, are crinkled along the midrib, with uneven development of halves of the blade, and with whitish patches at the margins; other leaves may be small and stipule-like. The internodes are usually shortened, and a rosette of anomalous leaves frequently appears at the apex of the epicotyledonary axis.

This paper reports the results of tests to determine the effect of different lengths of periods of after-ripening on the morphological development of the seedlings.

Material and methods

There is much variation in the behavior of peach seeds from different varieties and sources. With this in mind, pits[2] were secured from nineteen varieties and twelve sources,[3] from three

[1] Journal Article no. 627 of the New York State Agricultural Experiment Station.

[2] In this paper the term "pit" is used to denote the stony pericarp and inclosed seed, and the term "seed" is used in its strictly botanical sense.

[3] The writers are indebted to the following for supplying peach seeds used in these studies: Dr. Omund Lilleland and Dr. E. L. Proebsting, Uni-

seasons, totaling twenty-nine lots, as follows: Ambergem, Belle, Champion, Early Crawford, Elberta (two sources), Halehaven, Ideal, J. H. Hale, Lovell (eight sources), McAllister, Muir, Naturals (three sources), October Elberta, Polly, Salwey, Sunbeam, USSR 106, USSR 557, and Ward Late. More than 10,000 seeds were used in the course of the investigation.

sand or moist peat moss. In some treatments the entire pit was used; in others, only the seed. The material was germinated in soil in the greenhouse (68° F.) and in 100-mm. petri dishes on moist filter paper in the laboratory (68° F.). Seedlings were grown in soil in 6-inch pots in the greenhouse.

For excised embryos, the methods described by TUKEY (5) were employed,

FIG. 1.—Dwarfed peach seedlings from seeds which germinated after 3–6 weeks of after-ripening. *A*, USSR 106, 3 weeks; *B*, Lovell 1, 4 weeks; *C*, Lovell 2, 6 weeks. Cf. fig. 2 for differences in varietal response following same treatment.

Conditions favorable to after-ripening were provided in a controlled refrigerator maintained at 34°–36° F., the seeds being held in containers filled with either moist

using both ½-oz. bottles and agar, and also filter paper in petri dishes. Most satisfactory results were secured with the agar cultures in bottles.

Results

EXPERIMENT 1: SEEDS AFTER-RIPENED 3–12 WEEKS AND PLANTED AT WEEKLY INTERVALS

Approximately 100 seeds from each of ten lots of pits were placed under condi-

versity of California, Davis, Calif.; Kelly Nursery Company, Fordsville, Ky.; Harry Malter, Greening Nursery Company, Monroe, Mich.; Dr. Stanley Johnston, South Haven, Mich.; Wm. Shaefer, Sparta, Mich.; Howard Maloney, Maloney Brothers, Dansville, N.Y.; R. L. Holmes, Jackson & Perkins Company, Newark, N.Y.; and Tennessee Nursery Company, McMinnville, Tenn.

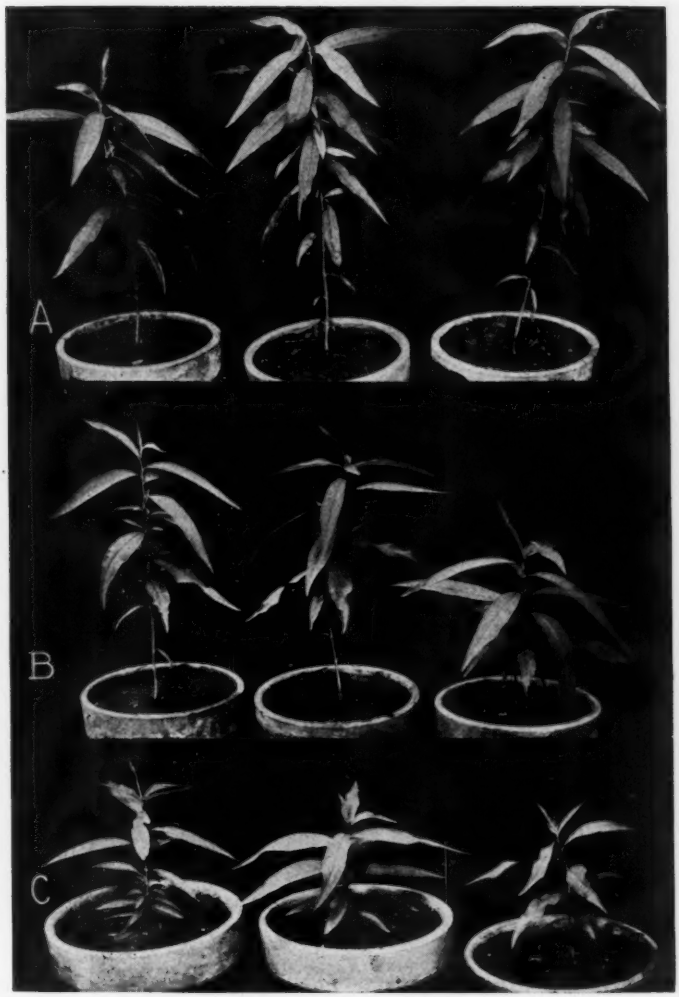

FIG. 2.—"Normal" peach seedlings from seeds which germinated after 3–6 weeks of after-ripening. *A*, Salwey, 3 weeks; *B*, McAllister, 4 weeks; *C*, October Elberta, 6 weeks. Cf. fig. 1 for differences in varietal response following same treatment.

tions favorable to after-ripening. Ten from each lot were removed at weekly intervals for 3–12 weeks and placed on moist filter paper in covered petri dishes. Of the seeds which germinated, representatives were planted in soil-filled pots and grown for 6–8 months in the greenhouses.

Some of the plants were dwarfed, with anomalous leaves (fig. 1); others showed no variations from typical vigorous seedling growth of well-after-ripened seeds (fig. 2). The dwarfed and anomalous plants from any given lot were most frequent among the samplings which were after-ripened for the shortest period of time. As the period lengthened, there were fewer such plants, until finally after 12 weeks there were none.

The frequency of occurrence of dwarfed and anomalous plants varied with the variety (figs. 1, 2). The variety Salwey, which appears to have a relatively short after-ripening requirement, produced no dwarfed plants, even with as little as 3 weeks of after-ripening. Belle and Champion, which appear to have an intermediate after-ripening requirement, produced both dwarfed and non-dwarfed plants throughout the 6-week period of after-ripening but produced only "normal" plants thereafter.

TABLE 1

DISTRIBUTION OF "NORMAL" AND DWARFED PEACH SEEDLINGS FROM SEEDS AFTER-RIPENED 3–12 WEEKS

VARIETY	PLANT TYPE	WEEKS OF AFTER-RIPENING									
		3	4	5	6	7	8	9	10	11	12
Belle	N*	1	1	1	.....	.....	.....	.....	3	.....	.....
	D	1	3	2	4	.....	.....	.....	0	.....	.....
Champion	N	0	2	3	2	5	.....	4	1	1	.....
	D	2	3	1	3	0	.....	0	0	0	.....
Lovell 1	N	.....	1	1	.....	.....	.....	.....	.....	4	.....
	D	.....	3	4	4	2	.....	4	1	1	.....
Lovell 2	N	.....	0	1	0	0	.....	0	.....	0	.....
	D	.....	1	1	2	1	.....	2	.....	1	.....
McAllister	N	4	8	.....	2	2	3	2	3	3	4
	D	.....	2	.....	1	0	0	0	0	0	0
October Elberta	N	0	1	1	5	2	3	1	.....	2	5
	D	1	1	1	0	0	0	0	.....	0	0
Salwey	N	3	4	5	8	8	9	7	.....	3	.....
	D	0	0	0	0	0	0	0	.....	0	.....
USSR 106	N	0	1	2	3	2	4	.....	1	2	4
	D	2	1	0	0	0	0	.....	0	0	0
USSR 557	N	0	.....	.....	.....	2	2	.....	1	.....	5
	D	1	.....	.....	.....	1	1	1	1	1	0
Ward Late	N	1	.....	1	1	.....	2	.....	.....	1	2
	D	.....	.....	.....	5	.....	.....	4	1	0	0

* N, normal; D, dwarfed.

The Lovell variety, which appears to have a relatively long after-ripening requirement, produced dwarfed plants even after 11 weeks of after-ripening.

The numbers of both dwarfed and normal plants for the different lots during the 12-week period are given in table 1. They show the after-ripening requirements for the production of normal plants for the other varieties not mentioned in the text.

Experiment 2: Excised embryos of non-germinating seeds after-ripened 3–12 weeks

Some of the seeds used in the preceding experiment, although apparently sound, failed to germinate. Since it has been shown that embryos from non-after-ripened seeds will germinate when excised, representatives of the non-germinating seeds were excised and grown

TABLE 2

Distribution of "normal" and dwarfed peach seedlings from embryos of excised seeds which failed to germinate after 3–12 weeks of after-ripening

Variety	Plant type	Weeks of after-ripening									
		3	4	5	6	7	8	9	10	11	12
Belle	N*	.....	0	0	0	0	1	.....	.....	.....	1
	D	.....	1	1	2	1	.....	1	.....	.....	1
Champion	N	.....	.....	0	.....	.....	.....	.....	0	.....	.....
	D	.....	.....	1	.....	.....	.....	.....	1	.....	.....
Lovell 1	N	.....	.....	.....	.....	0	0	0	.....	.....	0
	D	.....	.....	.....	.....	1	1	1	.....	.....	1
Lovell 2	N	.....	0	0	0	.....	0	0	.....	.....	.....
	D	.....	1	1	1	.....	2	1	.....	.....	.....
McAllister	N	.....	.....	.....	.....	.....	0	.....	.....	1	1
	D	.....	.....	.....	.....	2	1	.....	.....	0	0
Muir	N	.....	0	0	0	0	0	0	.....	0	2
	D	.....	6	1	5	4	4	3	.....	1	4
October Elberta	N	.....	.....	1	.....	.....	.....	.....	.....	1	1
	D	.....	.....	0	.....	.....	.....	.....	.....	0	0
Salwey	N	(All germinated; none required excising)									
	D										
USSR 106	N	.....	.....	.....	0	0	.....	0	0	.....	1
	D	.....	.....	.....	2	2	.....	1	1	.....	0
USSR 557	N	.....	0	.....	0	.....	.....	0	0	0	1
	D	.....	7	.....	1	.....	.....	1	2	1	1
Ward Late	N	.....	0	0	.....	0	.....	.....	1	.....	.....
	D	.....	1	1	.....	2	.....	.....	0	.....	.....

* N, normal; D, dwarfed.

to seedling size by the embryo-culture technique.

The results are shown in table 2. The lower number of individuals involved is due to the fact that most of the seeds germinated, leaving only a relatively few for the excising treatment. As in the previous experiment, some seedlings were dwarfed while others were typical of vigorous-growing seedlings from well-after-ripened seeds (fig. 3). Likewise, the frequency of anomalous forms was highest with seeds which had been after-ripened for a short period; it decreased progressively as the period of after-ripening lengthened.

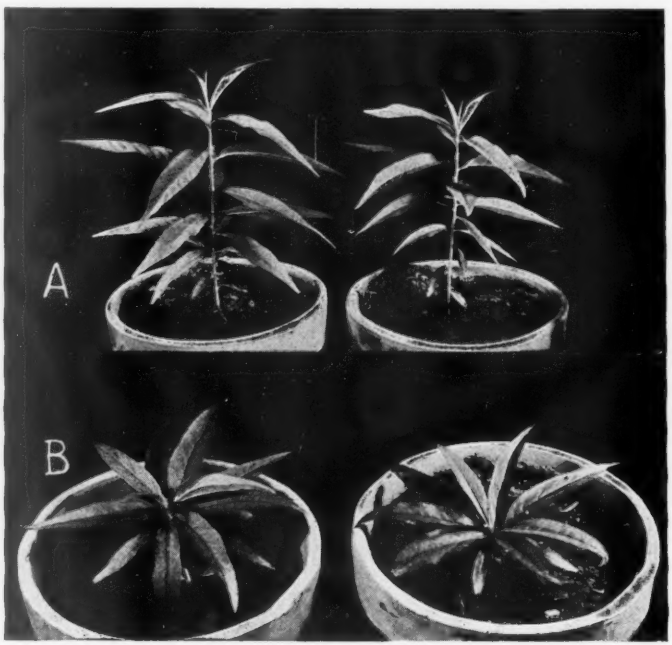

FIG. 3.—"Normal" and dwarfed seedlings from seeds of USSR 557 after-ripened for 5 weeks, showing specific after-ripening requirements for individual seeds of the same variety. *A*, normal seedlings from seeds which germinated after 5 weeks of after-ripening; *B*, dwarfed seedlings from seeds which failed to germinate until embryos were excised and cultured.

A comparison of tables 1 and 2 shows that the frequency of anomalous plants is higher from the excised than from the non-excised seeds following the same length of after-ripening. Thus, with variety USSR 106 only normal plants were observed from seeds which germinated after 5 weeks of after-ripening, whereas with seeds which did not germinate unless excised, the dwarfed forms appeared even after 10 weeks of after-ripening.

EXPERIMENT 3: EXCISED AND NON-EXCISED SEEDS AFTER-RIPENED 1–12 WEEKS AND PLANTED AT WEEKLY INTERVALS

Six hundred seeds each of the varieties Elberta and Lovell were placed in conditions favorable to after-ripening. Fifty of each variety were removed at weekly intervals for 1–12 weeks, in each

case ten being excised and forty non-excised. Each series of 100 was then planted in soil in pots in the greenhouse.

The resulting plants were similar to those reported in experiments 1 and 2. The frequency of dwarfed forms from both excised and non-excised seeds was relatively high for the shorter periods of after-ripening and became progressively lower as the after-ripening period lengthened.

The frequency of dwarfed seedlings was again higher from the excised than from the non-excised seeds following the same number of days of after-ripening.

Experiment 4: Successive lots of seeds after-ripened 1–12 weeks and then planted simultaneously

In the previous experiments, all seeds were placed in an environment favorable to after-ripening on the same day, and samples were removed at weekly intervals for 12 weeks and placed immediately in an environment favorable to germination. The question of daylength or of some other factor might conceivably have been introduced to affect the behavior of the seedlings, inasmuch as seedlings planted 12 weeks later than others were naturally subjected to a different daylength and probably to other factors. To avoid these possibilities, a test was conducted in which twelve lots of seeds were placed at successive and weekly intervals (1–12 weeks) in an environment favorable to after-ripening and were then all removed and planted on the same day. Two varieties were used, Lovell and Kentucky Naturals, and twenty-five seeds were used for each sample.

The results were similar to those from the previous experiments. Seedlings which developed from seeds after-ripened for relatively short periods (3–6 weeks) showed dwarfed and anomalous leaf characters, whereas seedlings from seeds after-ripened for longer periods (10–12 weeks) showed no such characters.

Experiment 5: Partially after-ripened seed

Two lots of Lovell pits were secured from commercial sources in California. They had been exposed out-of-doors in the vicinity of Davis and so were somewhat after-ripened. When the pits were received, some in one lot had already cracked, and some seeds were germinating, the radicles being 5–10 mm. in length. The germinating seeds were separated from the non-germinating seeds in uncracked pits, and twenty of the former and thirty-five of the latter were planted in the greenhouse in soil.

All twenty of the germinating seeds developed into seedlings typical of those from well-after-ripened seed. On the other hand, only seven seedlings developed from the thirty-five non-germinating seeds, and all seven were dwarfed, with anomalous leaves.

Loss of anomalous characters

The dwarfed and anomalous growths only rarely occurred elsewhere than in the epicotyledonary axis and its appendages. Occasionally a lateral shoot developed with anomalous leaves, terminating in a rosette, but this was the exception. However, new shoots arising from axillary buds showed no anomalous growth characters (figs. 4, 5). Further, when dwarfed plants were placed in a cool room (40° F.) for a month, so that they were checked in growth, and then brought into the greenhouse (65° F.), the new growth failed to show anomalous characters.

Discussion

The results from after-ripening suggest a relation to the growth patterns secured from culturing immature embryos in artificial media (**6**). Thus, no development may occur in peach embryos excised earlier than 51 days after full bloom when grown on 0.6% agar with 0.5% glucose and salt mixture T. Embryos excised 51 days after full bloom may show spreading and greening of the cotyledons, and small, white epicotyledonary leaves; 73 days, green cotyledons, hypocotyl 2–4 mm., epicotyl 1–2 mm., terminating in a rosette of small stipule-like appendages; 80 days, roots 10–20 mm. in length, epicotyledonary axis 7–15 mm., surmounted by a rosette of white, stipule-like appendages; 87 days, vigorous root development, central axis of epicotyl 20–22 mm. in length, terminated by a rosette of green stipule-like appendages; 94 days, vigorous root development, central axis of epicotyl 25–30 mm., terminated by rosette of small, green stipule-like appendages, occasional peachlike but malformed leaves; 105 days, vigorous root formation, stem 40–50 mm. in length, terminated by rosette of small, green stipule-like leaves, with peachlike leaves along the stem; 108 days, root and shoot development but leaves often broad and crinkled and plants dwarfed; and 118 days, root and shoot development but dwarfed plants.

The experiments reported here also suggest a relation to nutrition. Some of the anomalous growths are not unlike those secured from mineral deficiencies (**2**). It may be that during the after-ripening process certain materials are made available.

There is apparently a relation to vernalization indicated in the results, in which the subsequent performance of plants may be markedly altered by low-temperature treatment of the seeds. LYSENKO (**3**) has considered the developmental processes in a plant to be made up of individual steps and stages which proceed in strict sequence. A given stage cannot proceed until the preceding stage has been completed, and different stages require different external conditions.

FIG. 4.—Terminals of dwarfed seedlings, showing nature of abnormality: *A*, *A*¹, epicotyledonary axis terminating in rosette of anomalous leaves; *B*, *B*¹, normal shoots arising from axillary buds. (Note in *B*¹ the misleading appearance of "recovery" of the terminal bud from the dwarfed condition, whereas the new normal growth really arises from a lateral bud.)

Vernalization and after-ripening are similar, in that they are both physiological preconditioning treatments which significantly affect subsequent behavior of the plant.

In the case of peach seedlings, the anomalous growth is confined to the epicotyledonary axis and its appendages, and new shoot growth from axillary buds only rarely shows dwarfed characters. A study of the epicotyl during after-ripening is being made to determine whether morphological changes accompany the process.

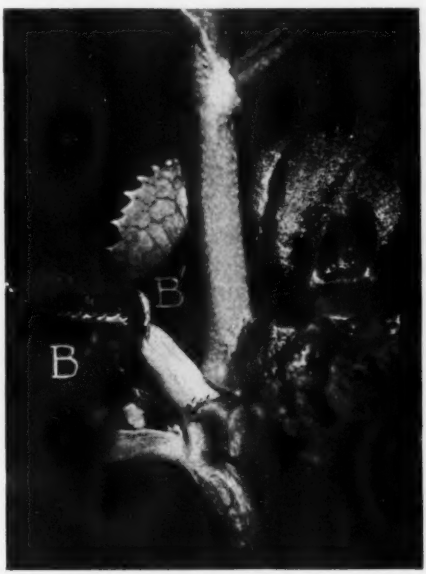

FIG. 5.—Apex of dwarfed seedlings which terminated in a rosette of anomalous leaves at *B*. "Normal" shoot (B^1) arising from axillary bud and assuming ascendency gives the misleading appearance of "recovery" of terminal bud from dwarfed condition.

The importance of complete after-ripening of fruit-tree seeds in horticultural enterprises, as in the production of fruit-tree seedlings, has not been determined. However, a high percentage of dwarfed and anomalous plants has been observed among sweet cherry seedlings which were suspected of having been subjected to insufficient after-ripening.

Summary

1. More than 10,000 peach seeds, involving nineteen varieties and twelve sources, were after-ripened at 34°–36° F. for weekly intervals ranging from 1 to 12 weeks, and then germinated and grown to seedling size at 68° F.

2. Some of the plants which developed were dwarfed, with anomalous leaves, resembling plants grown from excised embryos of non-after-ripened seeds. Such anomalous plants were most frequent among samplings which were after-ripened for the shortest period of time; they decreased progressively as the after-ripening period lengthened, until all seedlings were "normal."

3. The frequency of anomalous plants was higher from non-germinating, excised seeds than from germinating, non-excised seeds following the same length of after-ripening.

4. The frequency of anomalous plants varied with the variety.

5. Anomalous forms are not the result of photoperiod alone, inasmuch as both normal and dwarfed seedlings developed from seeds planted the same day.

6. Of two lots of pits from California, some were germinating and some not, when received. Of the germinating seeds, all developed into normal seedlings; only a few of the non-germinating ones developed, and these were all anomalous.

7. The dwarfed and anomalous growth of seedlings only rarely occurred in parts other than the epicotyledonary axis and its appendages. New shoots induced from axillary buds were free from dwarfed and anomalous characters.

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A RAPID EXTRACTION METHOD FOR FREE AUXIN AND ITS APPLICATION IN GEOTROPIC REACTIONS OF BEAN SEEDLINGS AND SUGAR-CANE NODES

J. VAN OVERBEEK, G. DÁVILA OLIVO, AND ELBA M. SANTIAGO DE VÁZQUEZ

Introduction

In a study of the growth and development of sugar cane (**18**), it became desirable to determine its auxin content. Various methods for the determination of the free auxin of plant tissues have been described in the literature (**19**). Among the most suitable methods for the extraction of free auxin are those in which heat is employed in order to prevent, during the extraction, liberation of auxin from bound forms or precursors. Heat is alleged to destroy an enzyme system responsible for this liberation or activation (**5, 13**). The immersion of sugar-cane material in boiling water for a brief period stopped the continued yield of auxin during the process of prolonged extraction.

A complication arose, however, when it became evident that as a result of the heating a substance was liberated from the cane material which has an inhibitory effect on the curvature of the *Avena* test. This substance is ether-soluble and is therefore extracted together with the auxin. It thus became necessary to find a more suitable method of extraction, which does not involve heating of the plant material. Since previous research (for literature, see **19**) has shown that the liberation of free auxin from inactive forms is a slow process, it seemed that free auxin, exclusive of that activated in the course of the extraction, could be removed from the plant by an extraction period of short duration. With this in mind, a study was made of the rate at which auxin was removed from fresh sugar-cane material during extraction with ether. The curves obtained indicated that large amounts of auxin are removed during the first half hour of the extraction, and relatively little later on.

To find convincing evidence that this first gush represented free auxin, a series of rapid extractions were made from the upper and lower sides of geotropically stimulated bean seedlings. It has been known for some time that geotropically stimulated seedlings yield more free auxin on the lower than on the upper side, as determined by the diffusion technique (for literature, see **21**). For this reason it is assumed that the lower side contains more free auxin than the upper. By using a rapid-extraction technique, a difference in auxin content between the two sides of

the stimulated bean seedlings was found which corresponded exactly with that obtained previously by means of the diffusion method. It seems, therefore, that the rapid-extraction technique yields a valid quantitative estimate on the free auxin of fresh material.

General methods

The sugar-cane variety most often used in these experiments was P.O.J. 2878, a variety developed in Java (P.O.J. is an abbreviation for Proefstation Oost Java—Experiment Station, East Java). It ranks among the more important cane varieties in Puerto Rico (7). The nodes were often used for trials on extraction techniques because they contain more auxin than the rest of the stalk (fig. 1). For the experiments with bean seedlings, Bountiful variety stringless beans (Kilgore) were employed. The seeds were germinated in sand in a darkroom and the stems used about 5 days after planting.

Before the auxin was extracted, the fresh material was cut into pieces about $5 \times 2 \times 2$ mm. and frozen by solid CO_2 whenever this was available. As an extraction solvent, purified diethyl ether was employed. The extract was reduced to a few cubic centimeters and poured on top of a known amount of hot agar in a vial 22 mm. in diameter and 48 mm. in height. The use of this larger size of vial constitutes a simplification in technique over that previously employed (16), in that it allowed the ether to be poured directly from the flask onto the agar. With the smaller vials previously used, the ether had to be carefully added, drop by drop, by means of a pipette to the hot agar in order to prevent spilling.

When the ether has evaporated, the auxin is automatically dissolved in the agar. This was subsequently poured into molds, and the resultant agar plates were then divided into sixteen small blocks ($3.5 \times 3.5 \times 1.4$ mm.), which in turn were applied to the decapitated plants in the *Avena* test.

The general principles of the *Avena* test (21) were followed, with the following modifications:

1. Owing to the even temperature and humidity at Mayagüez, as compared with more northerly climates, it was found that no special equipment for their control was necessary. Records of the thermograph showed that in the darkroom the average temperature was about 78° F. and that it rarely varied more than 2° during any 24-hour period. The humidity, although more variable than the temperature, could as a rule be maintained at 80% during the actual tests. This is the most desirable humidity for the *Avena* test when the plants are grown in sand.

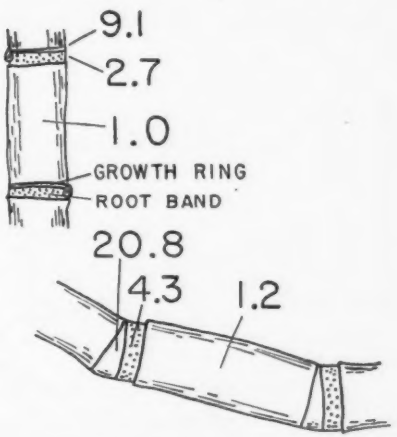

FIG. 1.—Distribution of free auxin (indoleacetic-acid equivalents γ/kg. fresh weight) as determined by rapid extraction ($2 \times \frac{1}{2}$ hr.) with ether, in vertical and horizontal sugar cane (cane in horizontal position for 4 days). Plant material frozen in dry ice prior to extraction. The leaf is attached just below root band.

2. Very satisfactory results (20) could

be obtained by planting the hulled and germinated oat seedlings (Victory variety) in moist sand in zinc boxes (20 × 3 × 3 cm.) instead of in the usual glass holders. The seedlings were planted more closely than is customary in the test in which glass holders are used. This allowed a certain degree of selection.

3. In earlier experiments, an interval of 1½ hours between the first and second decapitation was used, as is customary in the "standard" *Avena* test (21); later this period was extended to 3 hours, as suggested by SCHNEIDER and WENT (9).

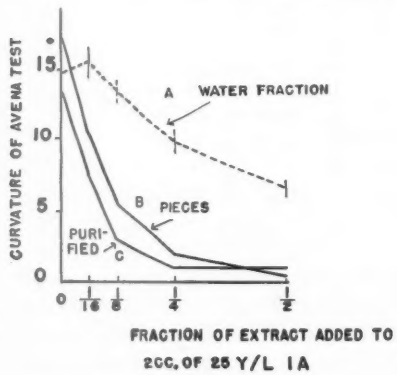

FIG. 2.—Depressing effect on auxin curvatures of *Avena* test by extracts from basal notes of sugar cane boiled 10 minutes in water (quantity "½" on abscissa corresponds to 50 gm. fresh weight of nodes). *A*, ether extract of water fraction of boiled nodes. *B*, ether extract of residue of boiled nodes. *C*, same as *B* except that fats and waxes were removed from extract by refluxing with petroleum ether; ether extraction lasted over-night. Vertical lines of *A* indicate magnitude of standard error of *Avena* test (n = 20–29).

The concentration curves obtained with synthetic indoleacetic acid under the conditions just described were similar to those obtained under the conditions of the standard *Avena* test in Pasadena, California. Here, as well as there, two general types of curves were obtained: (*a*) those showing a direct proportionality between auxin concentration and curvature, and (*b*) those inflected at the lower end and therefore showing a threshold (15). No periodic changes in sensitivity of the *Avena* test to a given amount of indoleacetic acid has been found so far. A more detailed account of the test and the auxin-extraction technique under tropical conditions has been given elsewhere (20). The magnitude of the standard error of the *Avena* tests may be judged from figure 2.

Results

SUBSTANCES ANTAGONISTIC TO AUXIN

Heating of plant tissues, followed by extraction with ether, has been recommended (5) as a method of extracting free auxin. When the nodes of sugar cane were thus treated, unsatisfactory results were obtained. Auxin yields were low and the results were not reproducible. In addition, if auxin was obtained it was found impossible to get reasonable concentration curves. In an attempt to investigate this unusual behavior, it was decided to add the extracts of boiled cane nodes to agar containing a known amount of indoleacetic acid. It was found that, as more of the extract was added to a given amount of indoleacetic acid, it depressed more the action of this auxin in the *Avena* test. In one test, for example, when agar containing indoleacetic acid in a concentration of 50 γ per liter was tested by itself, it caused a curvature of 15.4° in the *Avena* test. When, however, an amount of extract equivalent to 8 gm. fresh weight of cane nodes, which were submerged in boiling water for 3 minutes prior to ether extraction, was added to 2 cc. of this auxin agar, the curvature was reduced to 9.8°; and when extract of 33 gm. fresh

weight of boiled cane node was added, the action of the indoleacetic acid was almost entirely blocked.

Some extracts have been reported (11) which cause a "positive chemotropic" curvature in the *Avena* test; that is, a curvature opposite in direction from that caused by auxins. Extracts of boiled sugar-cane material when taken up in plain agar showed no evidence of inducing such positive chemotropic curvatures in the *Avena* test. Neither did preparations of the inhibitor cause bud inhibition when applied to the apical surface of vertical seed-pieces of cane.

In an attempt to separate the inhibitory substances from the auxins, the water fraction was filtered from the remaining pieces of cane immediately after boiling. Both fractions were extracted over-night with ether, and various portions of these extracts were added to 2 cc. of agar which contained 25 γ indoleacetic acid per liter. The results show that both the water and the solid fractions contain the inhibitory substance. However, the solid fraction yielded larger amounts (cf. curves *A* and *B* in fig. 2).

In further fractionation attempts, the ether extract of boiled cane nodes was evaporated to dryness, then water was added, which in turn was extracted twice with petroleum ether by boiling under reflux for one-half hour. The petroleum ether removed the lipoidal material, including the waxy substances, but the greater part of the inhibitory activity remained in the water fraction (fraction insoluble in petroleum ether), as shown by curve *C* in figure 2. In this respect the inhibitor resembles auxin. The fact that an extract from which the waxy substances have been removed still shows most of the inhibitory activity eliminates the possibility that these waxy substances in some mechanical way prevented the auxin from entering the *Avena* test plant and that this would account for the depressing effects of the boiled cane extracts.

Rate of auxin extraction as a function of time

The rate at which auxin is extracted from sugar-cane nodes varies considerably during the course of the extraction (20). This is again demonstrated in figure 3, which shows that during the first half hour of the extraction a relatively large amount of auxin is released from the tissue, while later considerably smaller amounts are yielded during similar periods of time. The technique consisted of cutting the nodes into small pieces and placing them in Erlenmeyer flasks containing purified ether. Some samples were frozen in solid CO_2 prior to being extracted. During the extraction the flasks were occasionally shaken, and after one-half hour the ether was decanted. The residue was washed twice with fresh ether and then extracted for another period of one-half hour. This process was repeated until four extractions of one-half hour each were made. Then

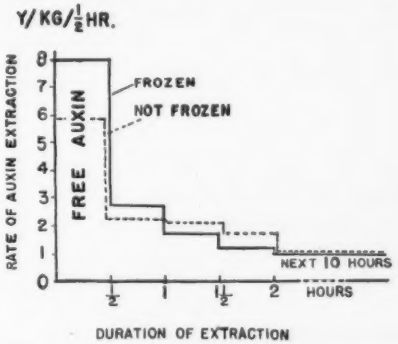

FIG. 3.—Rate of auxin yield from fresh sugar-cane nodes during extraction with ether. High initial yield is considered to represent principally the free auxin of tissue at beginning of extraction.

a fifth extract was made which lasted for 10 additional hours, and a sixth which lasted for 10 additional days. A number of experiments were conducted during the course of a year, the results of which agreed with the one presented previously (20) and with table 1 and figure 3 of the present paper.

Preliminary freezing in dry ice promoted the liberation of auxin during the first period. Crushing of the frozen material in a mortar did not materially increase the auxin yield over that of material only frozen.

The gush of auxin released during the first hour suggests that this represents most, if not all, the "free auxin," while the auxin which is liberated gradually represents auxin released from inactive or bound forms during the extraction. This release of auxin at a slow rate by the tissue may continue for many months in sugar cane (20), as well as in other tissues (5, 13). It cannot be due to insufficient removal of auxin during the first extractions, since freezing and grinding of the tissue does not alter the nature of the slow extraction (5, 13).

TABLE 1

AMOUNTS OF AUXIN (INDOLEACETIC-ACID EQUIVALENTS γ/KG. FRESH WEIGHT) EXTRACTED FROM SUGAR-CANE NODES DURING PROLONGED EXTRACTION WITH ETHER. ENTIRE EXPERIMENT RUN IN DUPLICATE

	NUMBER AND SEQUENCE OF EXTRACTIONS					
	1	2	3	4	5	6
Duration of extraction (hours)	½	½	½	½	10	240
Material frozen in dry ice	7.8	2.4	2.6	0.9	24.0 (1.2)*	72 (0.15)
	7.6	2.9	1.3	0.8	14.4 (0.7)	98 (0.20)
Material frozen in dry ice and ground	9.2	1.8	2.4	2.3	24.0 (1.2)	91 (0.19)
	7.2	3.6	1.2	1.0	22.8 (1.1)	90 (0.19)
Material neither frozen nor ground	5.0	1.9	2.2	2.4	20.4 (1.0)	100 (0.21)
	6.8	2.6	2.1	1.0	.....	.....

* Figures in parentheses are rate of extraction per ½ hour.

RAPID EXTRACTION METHOD AND AUXIN DISTRIBUTION IN GEOTROPICALLY STIMULATED BEAN SEEDLINGS

It should be possible to test the theory that the gush during the first half hour of the extraction represents free auxin, as one would conclude from the shape of the curve of figure 3. It has been conclusively demonstrated that more auxin can be obtained by diffusion from the lower side of geotropically stimulated seedlings than from the upper side. This by its very nature is free auxin. Since more free auxin diffuses out of the lower side, it may be assumed that this side contains more free auxin than the upper. It was therefore decided to extract, by the rapid method, the upper and lower halves of the hypocotyls of geotropically stimulated etiolated bean seedlings. The seedlings were cut off at the base and pinned on a horizontal board by means of cork disks. For 24 hours they remained on this board in a horizontal position, in the dark and in a saturated atmosphere. At the end of this period the cotyledons were removed and the hypocotyl split lengthwise into upper and lower portions.

The portions were then weighed and dropped in separate Erlenmeyer flasks containing purified diethyl ether. In some experiments the material was frozen in dry ice prior to extraction. Three extractions were made, each lasting one-half hour. As a rule, a fourth extraction lasting 24 hours was made. All operations took place in the darkroom, with the exception of the decanting and the readdition of ether.

The results, summarized in table 2, show clearly that by means of rapid extraction 67% of the total auxin is obtained from the lower side and 33% from the upper. This is in striking agreement with the results obtained by diffusion, which show (**21**, p. 165) that from seedlings, on the average, 66% is obtained from the lower and 34% from the upper side. This strongly supports the view that the gush of auxin of figure 3 represents principally free auxin.

Table 2 also shows the results of *prolonged* extraction of the upper and lower halves of geotropically stimulated bean hypocotyls. It is obvious that the difference between the two sides, which was so striking during the early part of the extraction, has disappeared. This is entirely in agreement with the assumption that the auxin which is slowly released during the later part of the extraction represents auxin activated or released from a precursor or a bound form during the extraction itself. As compared with the first rapid extraction, the ratio between the upper and lower halves seems to be reversed. On the average, 45% of the auxin extracted during the 24 hours following the first rapid extraction was recovered from the lower side,

TABLE 2

AUXIN EXTRACTION FROM UPPER (U) AND LOWER (L) HALVES OF GEOTROPICALLY STIMULATED HYPOCOTYLS OF ETIOLATED BEAN SEEDLINGS

Test	First extractions 3 × ½ hour				Next extraction 24 hours			
	γ/kg./1½ hours		Relative		γ/kg./24 hours		Relative	
	U	L	U	L	U	L	U	L
1	6.4	12.0	35	65	5.6	4.3	57	43
2	8.0	11.7	41	59	.....	.....	.....	.....
3	4.1	7.7	35	65	3.9	2.8	58	42
4	2.2	7.9	22	78	5.8	4.9	54	46
5	4.9	7.6	39	61	3.7	2.8	57	43
6*	3.0	9.7	24	76	.....	.....	.....	.....
7*	3.0	10.5	27	73	14.0	12.0	54	46
8*	7.0	9.9	41	59	5.8	5.3	52	48
Average (extraction)	4.8	9.6	33	67	6.5	5.4	55	45
Average (diffusion) of data for seedlings in literature (21)	.....	.....	34	66	.....	.....	.....	.....
Rate of extraction γ/kg./½ hour	2.4		.....		0.13		.....	

* Material frozen in dry ice prior to extraction.

while 55% was obtained from the upper.

GEO-INDUCED AUXIN INCREASE IN NODES OF SUGAR CANE

The nodes show marked response to geotropic stimulation. A few days after wedge-shaped body measuring 1 cm. or more at the lower end. A study was undertaken of the auxin relations in these nodes. (For the anatomy of sugar cane, see 1.) The canes were geotropically stimulated by forcing part of the culms of a stool into a horizontal position by

TABLE 3

AUXIN OBTAINED BY RAPID EXTRACTION FROM SUGAR-CANE STEMS (NON-CULTIVATED JAPANESE VARIETY) IN VERTICAL (V) OR HORIZONTAL (H) POSITIONS. MATERIAL NOT FROZEN PRIOR TO ETHER EXTRACTION (1½ HOURS). DATA EXPRESSED IN INDOLEACETIC-ACID EQUIVALENTS (γ/KG.). UPPER OF EACH PAIR OF FIGURES IN H COLUMNS REPRESENTS AUXIN CONCENTRATION OF UPPER HALF OF GEOTROPICALLY STIMULATED STEM; LOWER, THAT OF LOWER HALF

	TIME AND POSITION									
	1 day		2 days		3 days		4 days		5 days	
	V	H	V	H	V	H	V	H	V	H
Growth ring	1.6	2.9 / 2.8 — 2.9	3.3	2.9 / 3.5 — 3.2	0.8	3.7 / 3.7 — 3.7	4.8	8.4 / 10.6 — 9.5	6.0	12.2 / 9.6 — 10.9
Average vertical	4.0						5.2	11.6 / 11.6 — 11.6	6.6	12.0 / 11.6 — 11.8
Difference H-V	1.3		−0.1		2.9		5.6		5.1	
Root band	1.8	1.8 / 1.7 — 1.8	3.3	2.6 / 2.3 — 2.5	1.2	1.7 / 2.1 — 1.9	3.4	6.0 / 4.8 — 5.4	5.0	6.0 / 7.2 — 6.6
Average vertical	3.2						3.2	4.2 / 4.6 — 4.4	4.8	5.0 / 4.8 — 4.9
Difference H-V	0.0		−0.8		0.7		1.6		0.9	
Middle of internode	1.1	0.6 / 1.3 — 1.0	2.6	2.3 / 2.3 — 2.3	1.2	1.1 / 1.2 — 1.2	2.8	3.0 / 2.8 — 2.9	4.6	2.7 / 2.2 — 2.5
Average vertical	2.4						2.6	1.8 / 2.0 — 1.9	3.4	2.0 / 1.8 — 1.9
Difference H-V	−0.1		−0.3		0.0		−0.3		−1.8	

the canes have been placed horizontally, the *growth ring* (the intercalary zone, shown in figure 1, and which is only a few millimeters thick when the cane is in the vertical position) will grow into a means of ropes. The entire plant remained intact—no leaves were removed and the stem retained its connection with the root system.

After the canes had been in a horizon-

tal position for a certain length of time, which varied between 1 and 7 days, these canes, together with the remaining vertical ones from the same stool, were harvested. The growth rings, root bands, and a section of the middle part of the internodes were removed from the younger portion of the stalk, excepting the youngest apical portion. In the horizontal canes these sections were also subdivided into upper and lower portions with respect to their position during geotropic stimulation. These various tissues were cut into small pieces, as previously described, and either directly extracted for a short time (table 3) or frozen in dry ice prior to the rapid extraction.

The results are summarized in tables 3 and 4, while figure 1 shows the results of a single rather typical experiment in which the tissue was frozen in dry ice prior to extraction. There is a striking increase in auxin concentration of the growth ring after canes have been in a horizontal position for a few days. This effect was evident in all tests, without exception. The effect is less obvious in the root band and may be absent in the internode.

The unilateral distribution of auxin, which is so characteristic for geotropically stimulated seedlings, seems to be entirely absent in sugar cane. Neither the growth ring, the root band, nor the internode showed any evidence of a higher auxin concentration on the lower side. Because of the excellent results obtained with rapid extraction in bean seedlings, it is unlikely that the method is responsible for this failure to find the seedling type of lateral auxin distribution in sugar-cane nodes.

TABLE 4

AUXIN OBTAINED BY RAPID EXTRACTION FROM VERTICAL (V) AND HORIZONTAL (H) SUGAR-CANE STEMS (VARIETY P.O.J. 2878). EXPERIMENTAL CONDITIONS AS IN TABLE 3, BUT MATERIAL FROZEN IN DRY ICE PRIOR TO RAPID EXTRACTION (2×½ HOUR) WITH ETHER

	Time and position					
	3 days		4 days		7 days	
	V	H	V	H	V	H
Growth ring	17.0	40.7, 26.6 } 33.7	9.1	26.4, 15.2 } 20.8	10.0	19.5, 22.6 } 21.1
Average V	12.0					
Average H	25.2					
Root band	3.8	5.8	2.7	4.3	3.3	9.3
Average V	3.3					
Average H	6.5					
Middle of internode	0.5	1.8	1.0	1.2	.....	.....
Average V	0.8					
Average H	1.5					

Chemical nature of extracted auxin in beans and sugar cane

A number of experiments were conducted to determine whether the auxin obtained by extraction from the various materials used in the present investigation is of the type of indoleacetic acid or of auxin-a or auxin-b. The principal tests were acid- and alkali-stability determinations; but for sugar cane, additional determinations of the diffusion coefficient were made. The results of the latter have been reported previously (**20**). All determinations without exception indicated that the auxin extracted is of the type of indoleacetic acid.

TABLE 5

Relative stability to acid and alkali of auxins from sugar cane and bean seedlings. Figures in horizontal rows are comparable and indicate auxin content of solutions of equal volume in degrees of curvature in Avena test

Source of auxin	Reaction of solutions during autoclaving		
	Neutral	NaOH 0.01 N	H_2SO_4 0.01 N
Synthetic indoleacetic acid	16.0	12.1	0.8
Purified, from sugarcane nodes	8.9	6.8	0.0
	5.2	10.8	0.8
	Neutral	KOH 1 N	H_2SO_4 1 N
Rapid extraction from etiolated bean hypocotyls	10.3	13.3	1.1
	12.1	10.5	+1.5
	12.8	12.0	0.5
Same, but by prolonged, following rapid, extraction	1.7	9.4	1.4
	0.0	5.6	+0.5
	5.5	13.9	0.0

The auxin extracted from sugar cane was purified by refluxing with petroleum ether for two periods of 30 minutes each. This treatment removed the lipoidal material but did not decrease the auxin content of the extracts. The purified extracts were made acid (to 0.01 N H_2SO_4), alkaline (to 0.01 N NaOH), or left neutral, and then autoclaved for 15–30 minutes at 15 lb. pressure. A control solution of synthetic indoleacetic acid was run for comparison. After autoclaving, the extracts were neutralized, made equal in volume, and tested for their auxin content. The same procedure was followed for the auxin extracted from bean seedlings, except that the purification with petroleum ether was omitted and stronger acid and alkali (1 N) were used. Table 5 shows that the auxin extracted from all materials is stable to alkali and unstable to acid. In this respect it resembles indoleacetic acid and not auxin-a, which is stable to acid, or auxin-b, which is destroyed both by acid and alkali (**6**). With respect to acid and alkali stability, there is no difference between auxin obtained by rapid extraction and that obtained by a subsequent prolonged extraction (table 5).

Discussion

This paper has dealt with two main points, the development of a practical method for the extraction of free auxin and a comparison between the auxin relations in geotropic reactions of seedlings and of grass nodes.

For extracting the free auxin from plant tissues, without extracting in addition an appreciable amount of auxin which is set free during the course of the extraction, there are three possible methods: (*a*) heating the plant material prior to extraction, (*b*) extraction at low temperature, (*c*) rapid extraction. Heating the plant material was shown to be undesirable because it caused the release of substances shown to be antagonistic to

auxin and therefore interfering with the *Avena* test. Extraction at low temperature was investigated on a previous occasion (**20**), when it was found that although low temperature (−5° C.) slows down the rate at which auxin is released during prolonged extraction, it nevertheless remains of considerable magnitude; and at the end of 3 months the same total amount was released by extraction at 24° C. as during −5° C. However, in combination with the rapid extraction technique this low temperature extraction may become useful.

The rapid extraction technique for free auxin makes use of the fact that liberation of auxin during prolonged extraction proceeds at a slow rate (**13, 20**). Especially when the extraction is preceded by a breaking-up of the cells of the plant tissue by means of dry ice, this method is simple and reliable. Results obtained by the diffusion technique, which unquestionably tests for free auxin exclusively, could be duplicated with the rapid extraction technique.

Techniques in which the auxin is rapidly extracted from plant tissues are by no means new. In the earliest extraction techniques, extraction was of short duration (**12**). They were, however, abandoned when it was found that exhaustive extraction was not obtained. Since a slow process of auxin activation (or perhaps a release of free auxin from a bound form) continues during the ether extraction, there is reason again to favor rapid extraction.[1]

More auxin can be extracted from the lower than from the upper side of horizontal bean seedlings by the rapid method, and this is evidence for the correctness of the assumption that largely free auxin is extracted by this method. The evidence became even more convincing when a comparison between the ratio of the yields of upper and lower halves showed that these ratios were the same in the data obtained by the diffusion technique and by the rapid-extraction technique.

There appears to be a fundamental difference in geotropic behavior between seedlings and grass nodes. Seedlings do not increase their rate of growth when stimulated geotropically when they are rotated on the horizontal shaft of a clinostat (**2, 8**); the nodes of grass seedlings do increase their rate of growth (**4**). The total amount of auxin produced by geotropically stimulated seedlings is not increased more than that of vertical seedlings (**3**), while in grass nodes there is considerable increase. In seedlings, more free auxin is contained in the lower than in the upper part of geotropically stimulated organs, while in grass nodes this effect is either very weak (**10, 17**) or totally absent, as seems to be the case with sugar cane.

There is also another point of difference which is of importance in this respect. In all seedlings there is marked response to applied auxin. In grass nodes, by contrast, auxin does not seem to cause a growth response. UYLDERT (**14**) reported not the slightest action of auxin on the growth of grass nodes. Auxin applications on sugar cane will promote the development of roots from the root band, and will inhibit the lateral buds (**18, 20**), but in no case has any auxin-induced increase in growth of the nodes per se been found. This indicates that a factor other than auxin is the controlling agent in the growth and geotropic reaction of

[1] After this manuscript had been completed, an article (WENT, F. W., Amer. Jour. Bot. **31**:597–618. 1944) appeared which reported that from the stem tips of tomato plants more auxin is extracted during the first hour than during subsequent hours. This is in complete agreement with the results obtained here with sugar cane.

the sugar-cane node, and that the CHOLODNY-WENT theory (21), which seems to explain so well the geotropism of seedlings, does not hold for the geotropism of the nodes of sugar cane.

Summary

1. When tissue of sugar-cane nodes is submerged for a few minutes in boiling water, a substance is released which suppresses the action of indoleacetic acid in the *Avena* test (fig. 2). The substance is soluble in water and diethyl ether but not in petroleum ether. It is therefore extracted together with auxin, and for this reason heating of such plant material—and perhaps of plant material in general—prior to ether extraction is an undesirable step in auxin extraction technique.

2. In the course of ether extraction, there is during the first half hour a gush of auxin (fig. 3), but soon the rate slows down to a mere trickle, which continues for many months (20).

3. When the auxin of the upper and lower halves of horizontal hypocotyls of bean seedlings is extracted, using only the auxin yielded rapidly during the early part of the extraction, 33% is obtained from the upper side and 67% from the lower (table 2). This is precisely the average auxin distribution found for free auxin as determined by the diffusion method (21).

4. When the auxin of the upper and lower halves of horizontal hypocotyls of bean seedlings is extracted, using only the auxin yielded slowly during the later part of the extraction, no such unequal yields are obtained (table 2). Apparently the auxin yielded during the early part of the extraction is the free auxin of the plant tissues.

5. A rapid-extraction technique for the extraction of free auxin consists in freezing the plant tissue in dry ice (crushing is unnecessary); extracting it in Erlenmeyer flasks in ether for two or three periods of ½ hour each at room temperature; taking the extract up in a known amount of agar and using it on the *Avena* test (plants grown in sand instead of the usual glass holders).

6. When the free auxin content of horizontal stems of sugar cane is compared with stems in the vertical position, after a few days in the horizontal position the auxin concentration in the meristematic region of the node (the growth ring) increases 100% and more (fig. 1; tables 3, 4). This increase is less marked in the root band and is absent in the middle portion of the internodes.

7. The unilateral distribution of free auxin so striking in horizontal bean seedlings is totally absent in sugar cane. In further contrast to bean seedlings, no growth response to applied auxin—other than promotion of root growth and bud inhibition—was found in sugar cane.

8. The auxin extracted from etiolated bean hypocotyls and from sugar cane appears to be of the type of indoleacetic acid rather than auxin-a or auxin-b, in respect to stability to hot alkali and lability to hot acid. Both the auxin yielded during the early part of the extraction and that yielded during the prolonged later part belong to the indoleacetic-acid type.

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MAYAGÜEZ, PUERTO RICO

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FLORAL MORPHOLOGY OF CHRYSOTHAMNUS NAUSEOSUS SPECIOSUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **567**

EDNA SNOW

Introduction

Considerable work has been done on the morphology of the Compositae (**2, 3, 4, 5, 6, 10, 13, 14, 15, 17, 18, 19, 20, 22, 23**) but very little on the dominant composite plants of the west. HALL and CLEMENTS (**11**) have given attention to the taxonomy, origin, and relationship of the genera *Artemisia* and *Chrysothamnus*. DIETTERT (**6**) made an extensive study of *Artemisia tridentata* Nutt. in which he describes flower and seed development and development of leaf and stem. The present work was undertaken to extend the information on the floral development, gametogenesis, and vascular anatomy of one of the more abundant species of *Chrysothamnus, C. nauseosus speciosus* (Nutt.) Hall & Clements.

The genus *Chrysothamnus* is divided by HALL and CLEMENTS (**11**) into four sections, twelve species, and forty sub-

species. *C. nauseosus speciosus* is included in the section Nauseosi, the only one in which the twigs are densely pannose-tomentose. The group is characterized by a dense coating of long, weak hairs, more or less impregnated with a resinous substance. *C. nauseosus* exhibits the greatest variety of form.

C. nauseosus speciosus is a shrub 2–6 feet high, broad and rounded, usually blooming through September and October, sometimes even up to December. The rounded flower heads, usually consisting of five yellow disk flowers, are massed in such great numbers that the patches of color have given the plant the common name of goldenbush. It is also known in the Great Basin area as rabbitbrush, because rabbits utilize the thickets as a place of refuge.

This plant is found chiefly in the Great Basin region, although it occurs to some extent in all the western states. It occupies large areas of desert or semidesert country, frequently in pure stands, often along washes, on plains, and on mountain slopes. The species is deep-rooted and unfitted to compete successfully with the grasses in the surface soil. Under ecologically favorable conditions it is replaced by sagebrush or wheatgrass, but becomes re-established when such cover is broken by destructive grazing.

As a potentially important economic plant, it was investigated by HALL and CLEMENTS (11), who found rubber in the cortex and medullary rays of all twelve varieties examined. They estimated that the amount of rubber present averages about 2.83% but in individual plants may be as high as 6.57%. HALL and LONG (12) estimated that 150,000 tons of wild rubber are available in the roots and stems of this species, but the scattered and isolated location of the plants would make extraction and processing almost prohibitive, except in case of extreme need.

MATERIAL AND METHOD.—The material used in this study was collected at Provo, Utah, beginning in March, 1943, and continuing each week until October 6 of the same year, and from August 18 through November 2, 1944.

Sectioning is difficult, since the bracts are covered with small clubshaped hairs which have considerable resinous substance within the cells. Resin ducts are regularly associated with the bundles and continue into the floral tube, although they do not occur with the bundles of the rest of the flower.

Formalin-acetic acid-alcohol killing and fixing agent was used exclusively because of its rapid penetration of the resinous tissues. The fixed buds were dehydrated by the tertiary butyl-alcohol method, imbedded in paraffin, sectioned, and stained in a modification of Flemming's triple stain. All sections were cut at 10 μ, with the exception of a few longisections for the study of megasporogenesis, which were cut at 12 or 15 μ. Cross and longisections were made of all the samples collected. All drawings, with the exception of figures 55 and 56, were made with a camera lucida.

Observations

FLORAL DEVELOPMENT

The leaf buds of *C. nauseosus speciosus* appear early in March, but the flower buds do not appear until August. Development of the buds and flowers is relatively rapid. There are no over-winter buds, and the whole process of bud formation up to fertilization occurs in a period of about 6 weeks. The collections of September 4 were the first in which the archesporial cell of the ovule had developed. At this time the microsporogenous cells were undergoing reductional divi-

sions. The pollen grains were mature about September 17, and the megagametophyte by October 6.

There are usually five disk flowers in a head, each in the axil of a bract. Ray flowers are absent. The appearance of the individual flowers is essentially acropetal, the lowest one first. The second differentiates very close to it, the third and fourth at about the same time, and the fifth somewhat later.

No evidence of flower primordia appeared until July 21. At that time there was a broadening of the stem tip, with no further change until about August 17, when the flower bud primordia began to appear. Each individual flower appears as a blunt protuberance in the axil of a bract on the broadened axis. Growth is more rapid in circumference than in length, thus broadening the primordium. Zonal growth occurs at the periphery of the protuberance. At the outer edge of this zonal portion the corolla differentiates first, followed by the stamens, then the pappus, and finally the carpels (figs. 4–7). The order of development of *Chrysothamnus* is similar to that of *Aster* and *Solidago* as reported by MARTIN (**17**), but differs from certain others as reported by COULTER and CHAMBERLAIN (**5**) and MERRELL (**18**), in which the order of differentiation is corolla, stamens, carpels, and finally pappus.

Large nectaries appear about the same time as the archesporial cell and develop almost simultaneously with the ovule. They are located at the base of the stamens adjacent to the style of the pistil.

The corolla lobes curve inward at an early stage. Their marginal cells interlock, bringing about a fusion of the lobes (fig. 12). The corolla remains sealed until forced open by elongation of the stamens (fig. 56), as also occurs in *Artemisia tridentata* Nutt. reported by DIETTERT (**6**).

VASCULAR ANATOMY

The stele of the stem consists of five collateral bundles (fig. 13). At the tip of the stem five fibrovascular bundles are diverged from the stele (figs. 14, 15). Each of these is related to a single flower, of which there are five. At the base of each flower the vascular trace is similar in structure to the vascular bundle in the stem, of which it is a continuation.

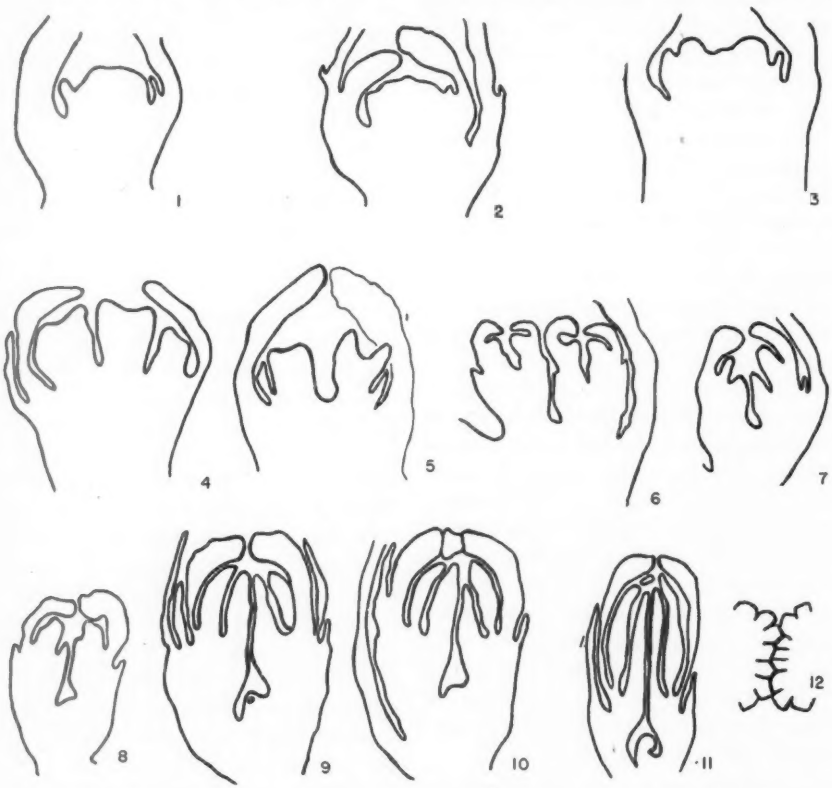
Just above this level in each flower there diverge from the solid stele two vascular bundles on the side farthest away from the central axis of the stem (fig. 14). At a slightly higher level three more bundles (figs. 15, 55*a–b*) are diverged.

These five bundles, equal in number to the corolla lobes, extend upward through the floral tube (figs. 16, 29) to the point of divergence of the stamen, where from each a single bundle diverges into each stamen (figs. 28, 29). This occurs at about the level where the pappus arises. There is no vascular supply to the pappus. The main course of the bundles is continued upward through the corolla, but instead of being opposite to the middle lines of the lobes—as in some flowers—they alternate with them to the point of their divergence. Here each is then separated into two equal branches, each of which traverses the near margins of two adjacent lobes (figs. 31, 32).

The five major vascular bundles may be numbered in a clockwise direction, number 2 being opposite the main axis of the stem. Numbers 4 and 5 diverged first from the central stele; the other three somewhat higher.

A single bundle supplies the ovule. This is a continuation of two bundles which arise as divergences from numbers 1 and 3 of the three major bundles just described. They are distinct in the funiculus but extend as a single bundle

through the raphe and curve over the chalaza of the very large ovule, extending down the single integument. The two stylar bundles diverge from bundles 2 and 5 just above the top of the ovule, the former at a slightly lower level than the



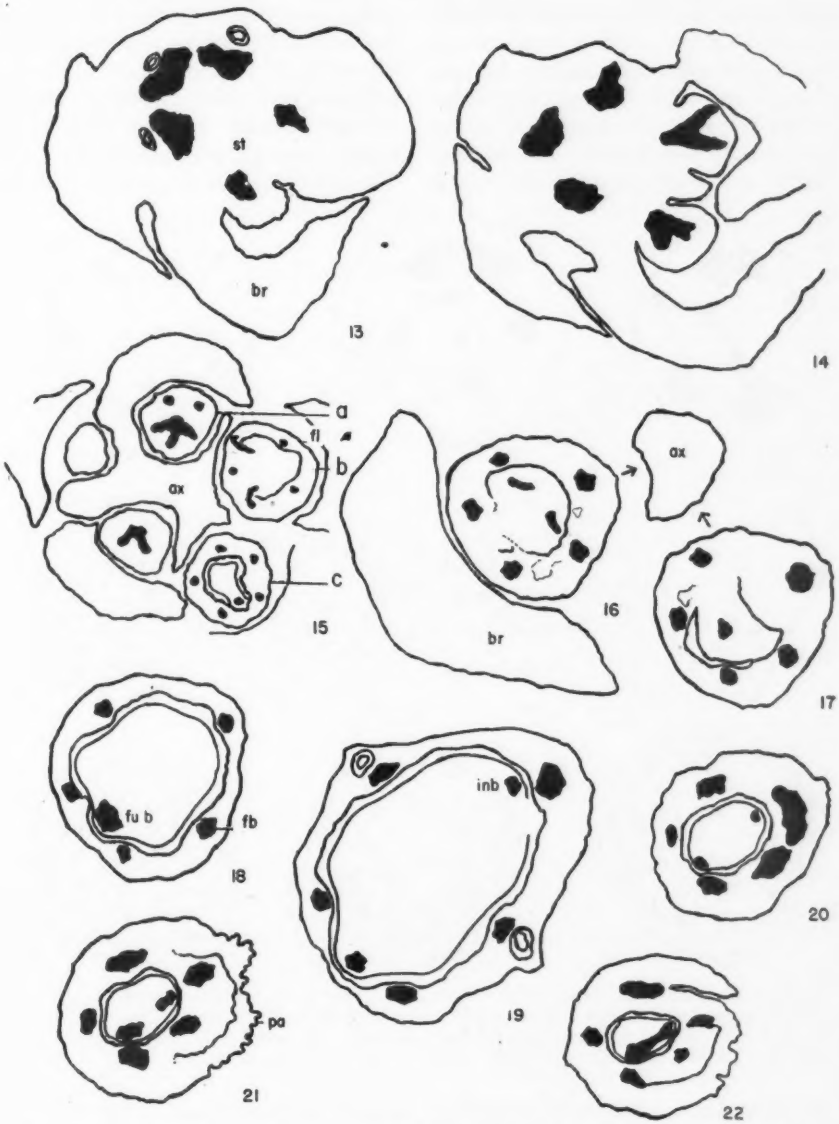
FIGS. 1–12.—Figs. 1–5, section of young flowering branch, showing beginning of heads. Figs. 6–10, early stages in development of flowers. Fig. 11, young flower showing relation of parts at archesporial cell stage. Fig. 12, portion of two corolla lobes, showing interlocking of marginal cells.

latter. These two bundles maintain their meristematic condition for a longer time than does the single bundle.

According to KOCH (14) the earliest mention of the venation of the corolla was by GREW in 1682, although the position of the veins was not discussed. The first clear description of the venation of the corolla was given by BROWN (1). *Chrysothamnus* is similar to the description given by him. The corolla of the primitive Compositae has a vascular supply of fifteen bundles—ten lateral, and five median (14, 15). The median vein of the petals is not present in *C. nauseosus speciosus*, there having been a reduction from this primitive condition to five bundles.

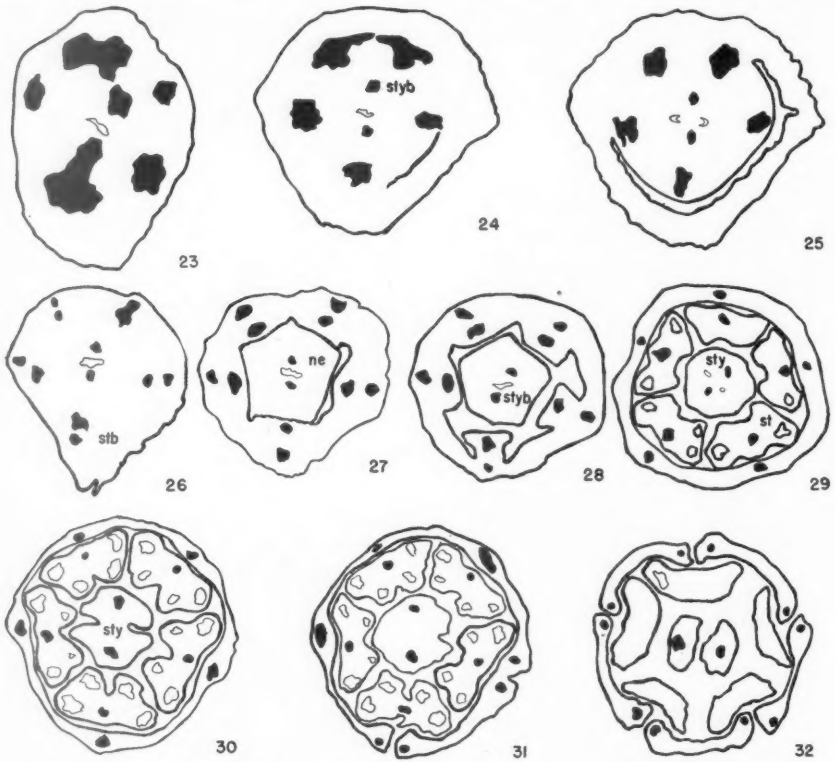
MICROSPOROGENESIS

The five stamens diverge immediately above the divergence of the corolla. The



FIGS. 13–22.—Transections at different levels of flowering branch, tracing course of vascular system upward into individual flower: Figs. 13–15, tip of stem axis showing divergence into individual flowers. Fig. 16, individual flower, showing five major veins and bract. Figs. 17, 18, five bundles of floral tube and vein of funiculus. Figs. 19, 20, five bundles of floral tube and veins of funiculus and integument. Figs. 21, 22, section near top of ovule, showing curving of vein over chalaza; pappus being cut off.

young stamen soon becomes distinctly four-lobed in cross-section, each lobe representing a microsporangium. In each corner of the anther a single large hypodermal cell differentiates as the archesporial cell, which soon divides periclinally and gives rise to a single layer undergoes periclinal divisions to form two layers. These later develop as a two-layered wall. The inner layer matures as a tapetum and consists of large, elongated cells, densely granular and usually having two or four nuclei (fig. 39*A*). These cells remain intact until after the



FIGS. 23–32.—Figs. 23–25, stylar bundles. Figs. 26–28, divergence of stamen bundles. Figs. 29, 30, stamen, stylar, and petal bundles. Figs. 31, 32, divergence of petal bundles to adjacent lobes. (*ax*, axis; *brt*, bract; *fl*, flower; *fb*, floral tube bundle; *int*, integument; *inb*, integument bundle; *nec*, nectary; *pb*, petal bundle; *rd*, resin duct; *st*, stamen; *stb*, stamen bundle; *sty*, style; *styb*, stylar bundle.)

of primary parietal cells and one layer of primary sporogenous cells. The primary sporogenous cell undergoes several successive anticlinal divisions, thus forming a group of four or five microspore mother cells.

Meanwhile the primary parietal cell meiotic divisions have occurred; then they become digested, disintegrated, and absorbed by the developing spores. A similar condition was found in *Silphium* by MERRELL (18).

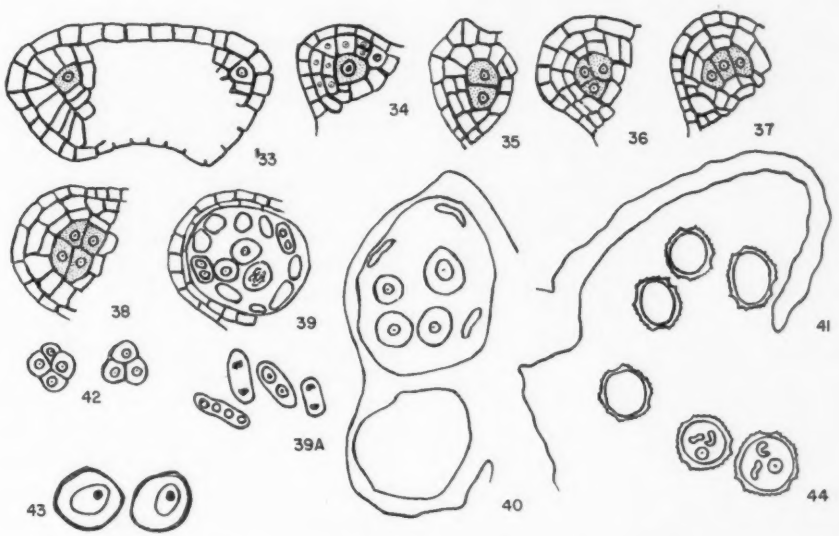
The microspore mother cells undergo meiosis (fig. 39), and the microspores

(fig. 43) are formed in tetrads (fig. 42). The tetrads split apart soon afterward, and after enlargement of the spore the nucleus divides to form a generative and a tube nucleus. The generative nucleus divides to form two spherical sperm nuclei which later elongate (fig. 44).

The mature microgametophyte in the anther is essentially spherical, having three furrows, and has a spinous wall

into the ovule instead of ending abruptly just below the edge of the funiculus. MERRELL states, "Whatever may be the condition in other Compositae, the ovule of *Silphium* as shown by its origin and its bundle relations is the termination of the floral axis, that is, the ovule is cauline."

Within the nucellar tissue the single hypodermal archesporial cell appears rather early and is easily recognized by



FIGS. 33–44.—Stages in development of anther: Figs. 33–38, sporogenous cells, primary parietal and tapetal layers. Fig. 39, pollen sac at microspore mother cell stage. Fig. 39A, cells of inner tapetal layer, showing two or four nuclei. Fig. 40, pollen sacs at microspore stage. Fig. 41, one-half mature anther, showing one pollen sac. Fig. 42, microspores, tetrad stage. Fig. 43, microspores. Fig. 44, microgametophyte at time of shedding.

(fig. 44). At this stage the sperm nuclei are elongated, are slightly crescent- to spindle-shaped, and lie free in the cytoplasm of the pollen grain.

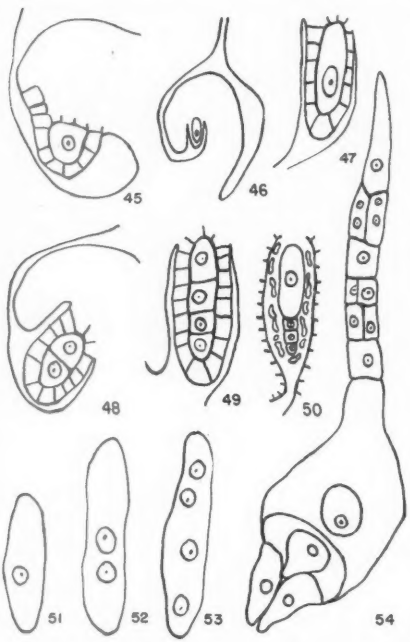
MEGASPOROGENESIS

The nucellus arises from the placenta at the tip of the axis and appears to be cauline. In the ray flower of *Silphium* (18) the axial bundle extends directly

its large size, granular appearance, and greater affinity for stains (figs. 45–47). The archesporial cell enlarges rapidly without dividing and becomes directly the megaspore mother cell. The elongated spore is covered by a single layer of nucellus. It follows the usual pattern of meiosis, forming a linear tetrad (figs. 48, 49). Three megaspores, usually those nearest the micropylar end, disintegrate

(fig. 50). The remaining one increases rapidly in size, as does the ovule, which soon fills the ovary cavity (fig. 51).

The functional megaspore undergoes three successive divisions (figs. 51–54), as has been reported previously for other Compositae (**4, 5, 6, 14**), forming at first a gametophyte of the eight-nucleate type. Wall formation occurs, dividing it into



FIGS. 45–54.—Stages in development of megagametophyte: Fig. 45, archesporial cell stage. Figs. 46–49, development of megaspores. Fig. 50, disintegrating megaspores (high power). Fig. 51, functional megaspore. Figs. 52, 53, development of megagametophyte. Fig. 54, mature megagametophyte showing synergids, egg, polar nucleus, and nine antipodal cells.

seven distinct cells. Subsequent divisions of the antipodals occur. The mature megagametophyte occupies about one-half of the entire length of the ovule. The lower two-thirds of the sac is narrow and elongated, but the central region of the upper one-third increases in breadth. None of the material examined showed fusion of the polar nuclei, which came to rest near the egg cell, but it apparently occurred early. The egg cell is pear-shaped, attenuated toward the micropylar end. The synergids, which are in close contact with the egg, are rather slender, with attenuated ends which extend into the micropyle. As already stated, the antipodal cells often divide, so that a variable number is formed. These differ in size and arrangement. Megagametophytes having six to ten antipodal cells were found, and some of these cells had more than one nucleus (fig. 55). The antipodals are arranged in a more or less linear row, although in several cases two cells lie side by side. Often such cells have been cut off in planes at right angles to each other. The antipodal cell farthest from the micropyle is usually the largest. It elongates rapidly, often occupying about one-third of the space of the antipodal tissue. These results agree closely with those of CHAMBERLAIN (**2**) and OPPERMAN (**19**), but are in contrast with those of MARTIN (**17**), who found no walls for these cells, and not more than four antipodal cells, and never in a single longitudinal row. The antipodal tissue persists very late in the development of the embryo.

GUIGNARD (**8, 9**) was one of the first to describe the increased development of the antipodals. He found them persisting and increasing in number up to ten uninucleate cells in *Conyza ambigua*. COULTER and CHAMBERLAIN (**5**) described as a common feature of the Compositae the enlargement of the distal antipodal cell and its penetration into the chalazal tissue. The nucellar cells adjacent to the antipodals are elongated and loosely connected. The antipodal cells are equal to—or exceed—the rest of the megagametophyte in length.

The nucellar tissue surrounding the two-thirds of the gametophyte nearest the micropyle begins disintegration almost simultaneously with that of the three nonfunctional megaspores, and few of its cells remain when the four-nucleate stage of the megagametophyte is reached. When the megagametophyte is mature this nucellar tissue has disappeared. Following the disintegration of the nucellar tissue, one or two inner layers of cells of the integument closely surround and are in contact with the megagametophyte, except its antipodal portion—which projects into the tissue of the ovule near the chalazal region. This tissue becomes digested and absorbed by the gametophyte. The cells of the integument are narrowly columnar, compact, densely granular, stain darker, resemble a secondary nutritive layer, and form the "epithelial" layer characteristic of the Compositae as described by HOWE (13).

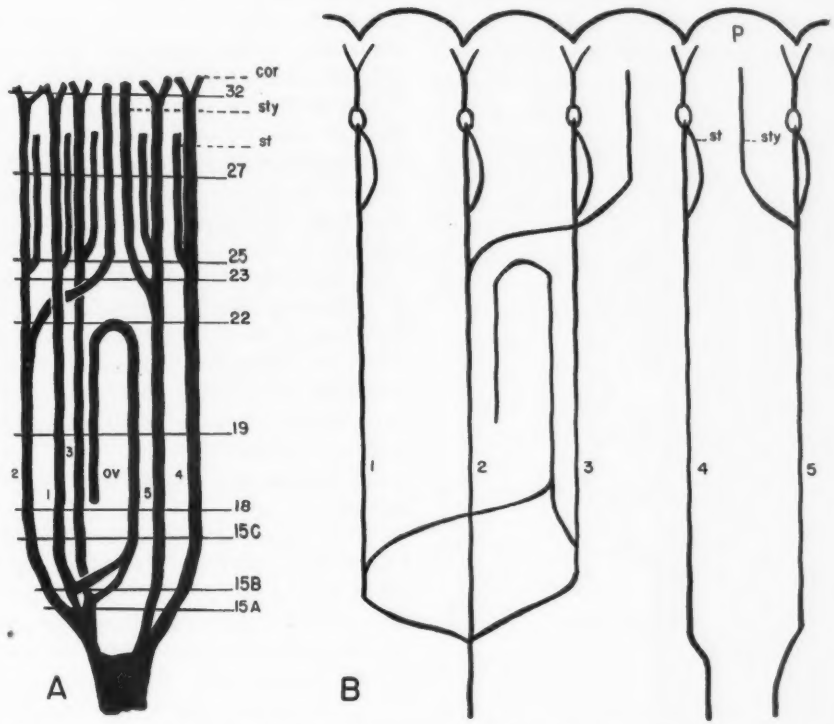


FIG. 55.—Diagram showing *A*, vascular anatomy of flower in longisection in three dimensions; *B*, same, in two dimensions.

Discussion

The development of the flowers and the vascular anatomy of *Chrysothamnus nauseosus speciosus* extends, and agrees somewhat with, the observations made by SMALL (22), CHAMBERLAIN (2, 3), MARTIN (17), KOCH (14, 15), MERRELL (18), OPPERMAN (19), BROWN (1), WARMKE (23), and GUSTAFSSON (10). In order of floral development it is like that of *Silphium*, in which the pappus appears

before the pistil, but unlike *Aster*, *Solidago*, and *Erigeron*, in which the pistil is the last structure to differentiate.

KOCH (14) describes the vascular system of the composite flower and divides it into four types. The fourth or discoid type which she describes is typical of *C. nauseosus speciosus*, and according to her it is the advanced type. In this type

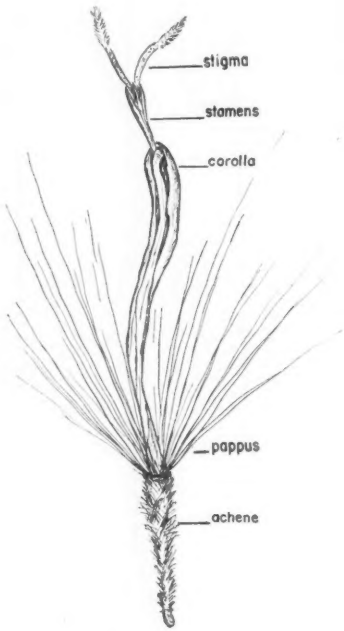


FIG. 56.—Old flower just prior to loss of corolla, stamens, and stigma.

there is an absence of midveins in the corolla lobes of the tubular flower. The presence of veins bordering the margins of the lobes is explained on the basis of the fusion of the lateral bundles of primitive Composites, and the loss of the median bundle. The five fused lateral bundles of the floral tube divide at the sinuses and border the corresponding lobes.

BROWN (1) describes this condition and states that the Compositae agree in two points: "1. Valvular aestivation, or the margins of segments dehiscing like capsule valves. This may be found in other families. 2. The nerves of the corolla are equal to the lobes, but instead of being opposite and passing through their axes as in other plants, they alternate with them, each divides into two equal branches at the top of the tube."

The absence of pith in the stele as it diverges into the individual flowers is rather an unusual condition. So far as the writer is aware, this feature is not mentioned in the literature on the Compositae.

The stylar bundles, diverging at slightly different levels from bundles 2 and then 5 (fig. 55*A–B*), bear out the work of SMALL (22). He states, "BROWN gives the orientation of the stylar bundles correctly as anterio-posterior and the ovarial bundles as lateral."

The vascular anatomy of a plant is especially important in explaining epigyny. The nature of the floral tube has been described by WILSON and JUST (24), and also by EAMES as reported by DOUGLAS (7). They state that there is abundant proof from the course of the bundles that the inferior ovary represents adnation in its extreme form. The vascular anatomy of *C. nauseosus speciosus* shows that the floral tube is not an invaginated axis but may be composed of undiverged basal parts of the flower. Certainly there is in *Chrysothamnus*, as well as in other Compositae, considerable zonal growth of all parts up to the top of the ovary (figs. 9, 10), and of the petals and stamen for a considerable distance above that level (fig. 11).

There seems to be general agreement that the Compositae are characterized in general by having male gametes existing as naked nuclei in the pollen grain,

elongated and curved, or irregular in shape (PODDUBNAJA [20] as reported by DIETTERT [6]).

One notable feature in *Chrysothamnus* is the relatively extreme size of the ovule. It grows rapidly after the archesporial cell stage. A representative set of measurements showed the gametophyte to be about 1.4 mm. and the ovule about 3 mm. in length, the latter equaling about one-fourth the full length of the flower. In my material no ovules were found with more than a single archesporial cell.

The antipodal cells vary in number, size, and position, as well as in number of nuclei. The usual number of three antipodals was not seen in this species. Discussions of the number and character of the antipodal cells in the Compositae have been given by COULTER and CHAMBERLAIN (5), SMALL (22), and SCHNARF (21). The enlargement and extension into the chalazal tissue is common and corresponds with the description of *Artemisia* reported by DIETTERT (6). Most investigators agree that the haustorial type of antipodals is concerned with the nutrition of the megagametophyte and that they aid either in digestion, or in conduction (or both) of substances to the gametophyte (8, 16, 20).

At the time the megagametophyte is developing, disintegration of some of the cells of the ovule occurs. These cells lie immediately interior to the epidermis of the ovule, principally in the chalazal region. The epidermis of the ovule remains intact and becomes thickened at the time the megagametophyte is mature, but the walls of the two layers of cells interior to it have been broken down. This seems to be a common occurrence in all the sections studied, but no mention of this disintegration has been found in the literature.

Summary

1. *Chrysothamnus nauseosus speciosus* (Nutt.) Hall & Clements is a potentially important economic shrub of western United States. It is 2–6 feet high, and is a subclimax dominant of the sagebrush-wheatgrass association.

2. It blossoms through September and October. The yellow flower heads are rounded, usually consisting of five disk flowers, with no ray flowers present.

3. The flower bud primordia appear during the latter part of August, and fertilization occurs the early part of October. The floral organs develop in the sequence: corolla, stamens, pappus, and pistil.

4. The marginal cells of the corolla lobes curve inward at an early stage, interlock, and bring about fusion of the lobes. Later the elongation of the stamens forces the flower open.

5. The fibrovascular bundles are equal in number to the corolla lobes but are alternate with their median lines rather than opposite. The stylar bundles are anterio-posterior, and the ovule bundle is lateral.

6. The mature microgametophyte at the time of shedding has three nuclei, a tube nucleus, and two elongated naked sperms.

7. A single archesporial cell was found in each ovule. It gives rise to four megaspores. The chalazal megaspore is the functional one.

8. The nucellus surrounding the mature megaspore mother cell and the four spores consists of one layer of cells. There is a single massive integument and a single large ovule. The inner layer of the integument is in contact with the megagametophyte and appears tapetal in nature.

9. The mature megagametophyte con-

sists of two synergids, an egg cell, two polar nuclei which fuse early, and a variable number of antipodals which show much diversity in their size, number, and arrangement, but are mainly linear. The number of antipodal cells varies from six to ten, each having from one to several nuclei. The cells are persistent and usually become haustorial in nature, penetrating the chalazal region and causing disintegration of the cells surrounding the region.

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RELATION OF TEMPERATURE TO THE SELECTIVE HERBICIDAL EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID

P. C. MARTH[1] AND F. F. DAVIS[2]

Introduction

In the course of previously reported investigations with 2,4-dichlorophenoxyacetic acid as a selective herbicide (**2, 3, 6, 9**), it was observed, under conditions in the field, that spray applications made in late fall and early winter were much slower in inducing plant responses than those applied during warmer periods. Herbicidal effects of 2,4-dichlorophenoxyacetic acid are to some extent dependent on the continuance of plant growth for a period of time following treatment. It is therefore to be expected that temperature may influence the rate of response and govern, to some extent, the time required to kill plants by this means. Furthermore, the effect of temperature is pronounced with applications of growth regulators other than 2,4-dichlorophenoxyacetic acid, and with plant-growth responses other than those of a herbicidal nature (**1, 4, 5, 7**).

The present experiments were conducted in the vicinity of Beltsville, Maryland. They were undertaken to determine the effects of temperature on the responses of some weeds to treatment with 2,4-dichlorophenoxyacetic acid or with its sodium salt. Some plants were kept at relatively low temperatures, others at medium, and others at high temperatures. The plants were subsequently treated and grown under these respective temperature conditions. Other plants were treated and grown for several weeks at a low temperature and then transferred to a higher one so as to induce more rapid vegetative growth. In addition, field applications of both the acid and its sodium salt were made in December on dormant plants of *Plantago lanceolata* in soil which was frozen to a depth of 2 inches. Subsequently, separate lots of the treated and the untreated plants were brought into the greenhouse at biweekly intervals, and their growth under temperature conditions (65° F.)[3] favorable to vigorous vegetative growth was observed.

METHODS.—In general, aqueous sprays containing 0, 500, 1000, and 1500 p.p.m. of 2,4-dichlorophenoxyacetic acid were applied to twenty-four or more plants of each species growing at the various temperatures. In preparing the sprays, the acid was first dissolved in sufficient Carbowax 1500 (**8**) to give a final concentration of 1.0% of the wax. Sprays containing 1000 p.p.m. of the sodium salt of the acid were prepared by dissolving the salt directly in a measured volume of water containing a small quantity of laundry soap to serve as a spreader. Carbowax was not used in the preparation of salt solutions. All spray applications were made with a 1-quart capacity compressed-air sprayer operating at 60-lb. pressure. A measured volume of spray was applied to each lot of plants, so that approximately the same coverage was obtained in each instance.

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[3] All temperatures throughout this paper are Fahrenheit.

Results

In an experiment initiated on November 16 and 17, several hundred 1-year-old plants of *Barbarea verna* (early winter cress) and *Plantago lanceolata* (narrow-leaved plantain) were transplanted from the field to 4-inch clay pots. The plants were then allowed to grow in a greenhouse at 65°–75°, for a period of 27 days in the case of *Barbarea* and for 43 days in the case of *Plantago*, during which time both species became well established and initiated new vegetative growth. At the end of this period, uniform plants that had developed leafy rosettes 4–6 inches in diameter were grouped into four lots of ninety-six plants for each species. One group of each species was then grown under each of the following temperature ranges: 32°–40°, 50°–60°, 65°–75°, and 75°–90°, respectively. The plants were held at each temperature level for 1 week prior to treatment.

All yellowed or otherwise discolored foliage was removed from the plants at the time of treatment. At intervals of 2–4 days thereafter, records were made of the number of leaves which subsequently turned yellow, the degree of epinasty, or the death of the plants in the different treatments. In the case of *B. verna* the rate of loss of green foliage color followed closely the rate of dying of the plants under the different spray treatments and temperature exposures, whereas with the other species the number of days required for complete killing was a more reliable measure of effectiveness of treatment than was leaf color change.

TABLE 1

EFFECT OF GREENHOUSE TEMPERATURE ON RELATIVE RATE OF KILLING OF BARBAREA VERNA FOLLOWING AQUEOUS SPRAY APPLICATION CONTAINING 500-, 1000-, AND 1500-P.P.M. CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID, AS INDICATED BY PERCENTAGE OF GREEN LEAVES REMAINING. CARBOWAX 1500 INCLUDED AT 1.0% CONCENTRATION IN EACH RESPECTIVE CONCENTRATION

Greenhouse temperature (° F.)	Percentage of original green leaves remaining at indicated number of days after treatment															Controls (44 days)
	4 days			14 days			24 days			34 days			44 days			
	500*	1000	1500	500	1000	1500	500	1000	1500	500	1000	1500	500	1000	1500	
75°–90°	86	87	87	25	23	23	3	3	0†	0	0	0	0	0	0	62
65°–75°	84	84	77	26	23	24	6	6	6	0	0	0	0	0	0	70
50°–65°	100	100	100	50	50	48	36	33	32	15	22	20	0	0	0	86
32°–40°	97	97	96	80	77	81	59	54	50	47	53	43	45	43	40	69

* Concentration (p.p.m.) of acid in spray.
† 0 indicates entire plant dead.

Barbarea verna

No significant difference in the rate of killing was observed at the two higher temperature ranges (65°–75° and 75°–90°), as shown in table 1. At both ranges the plants responded at about the same rate, and all were dead 34 days following treatment.

At a lower temperature (50°–65°) the plants remained alive significantly long-

er, and an interval of 44 days was required before all the treated plants were dead. None of the treated plants grown at 32°–40° were dead 44 days after treatment (figs. 1, 2), although they lost a higher percentage of leaves than did the controls during this period (table 1). At the end of 44 days, however, the untreated plants grown at 32°–40° had lost 31% of their old leaves and had produced little new growth, while at the next two higher temperature ranges the controls had grown rapidly, had become severely pot-bound, and had started to yellow because of decreased soil fertility.

No significant difference in effectiveness was found between spray applications of 500-, 1000-, or 1500-p.p.m. concentrations of the acid at any of the temperatures used (table 1).

In a comparable experiment, twelve plants of *B. verna* that had been treated with 1000-p.p.m. concentration of the acid and grown for a period of 35 days in the greenhouse at 32°–40° were removed to a warmer house (65°–75°), while twelve others were left at the lower temperature. At the time of shifting, the treated plants had developed some dead leaves (26%) and had stopped vegetative growth but were for the most part green in appearance. Following the change to the higher temperature, all twelve treated plants removed were dead 15 days later (fig. 1), while the treated plants that remained continuously at 32°–40° had changed but slightly in appearance 20 days later. Apparently the acid was present in lethal amounts within or on the surface of the treated plants, but under low temperature (32°–40°) conditions the responses associated with killing were not fully expressed, with the result that the plants remained alive over an extended period at the low temperature but died when subjected to a temperature ordinarily more favorable for growth.

Plantago lanceolata

An experiment was conducted in the greenhouse with *P. lanceolata* similar to that described for *Barbarea*. Plants treated with 2,4-dichlorophenoxyacetic acid at 500-, 1000-, and 1500-p.p.m. concentration and grown at 65°–75° were killed more quickly than comparable plants treated and grown at 75°–90°. The

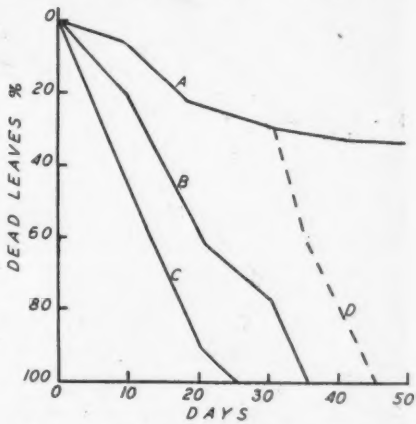

Fig. 1.—Relative rate of killing of *Barbarea verna* growing at different temperatures in the greenhouse following aqueous spray treatment with solution containing 1000-p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 1.0% Carbowax 1500: *A*, at 32°–40°; *B*, at 40°–50°; *C*, at 65°–75°; and *D*, at 32°–40° (then after 30 days the plants of this lot were moved to 65°–75° F.).

twenty-four plants included in each treatment at the former temperature were all dead at the end of 23 days, whereas those growing at the latter temperature required 31 days for complete eradication. The control plants grew more rapidly at the lower temperature than at the higher. As was the case with *Barbarea*, no significant difference in rate of killing was observed between the three concentrations of the acid that were applied at the various temperatures.

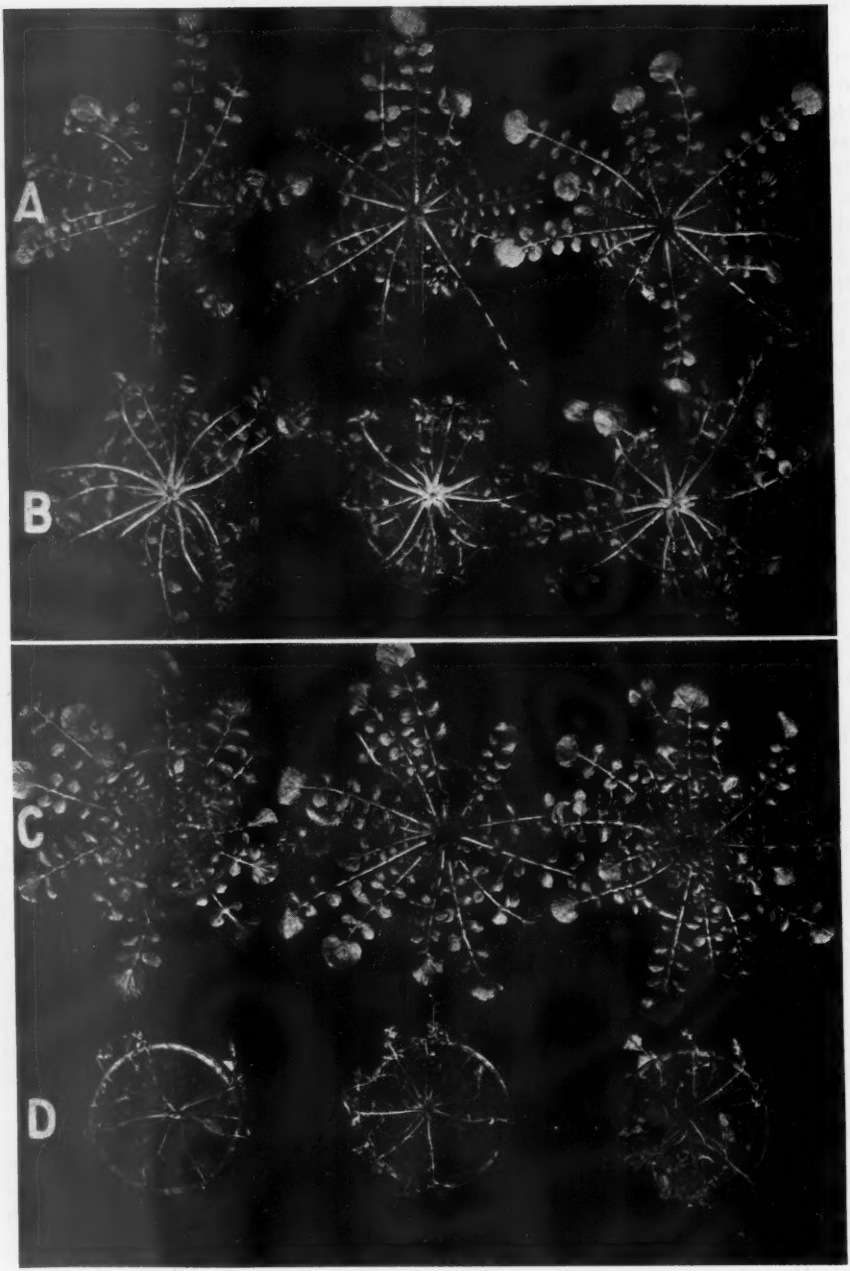

FIG. 2—Effect of aqueous spray containing 1000-p.p.m. concentration 2,4-dichlorophenoxyacetic acid and 1.0% Carbowax 1500 on killing of *Barbarea verna* treated and grown at relatively high and low temperatures in the greenhouse: *A*, unsprayed, 32°–40°; *B*, sprayed, 32°–40°; *C*, unsprayed, 65°–75°; and *D*, sprayed, 65°–75°. Sprays applied at rate of 5 gallons per 1000 sq. ft. on December 18; photographed 30 days later.

At the end of 21 days the treated plants growing in the greenhouse at 50°–65° showed much more epinasty and rolling of the leaf petioles than those growing at 32°–40°. However, the foliage on all plants at both temperatures was still green at this time. Twelve plants from each treatment were therefore moved from the two relatively low temperatures into a greenhouse at 65°–75°. Following this change, the treated plants previously growing at 50°–65° were all dead within a period of 9 days, while those moved from the 32°–40° range were dead after 14 days. These results further substantiate those obtained with *Barbarea*, that response to the acid is markedly reduced by low temperatures unfavorable for vegetative growth; furthermore, if the growth substance is in or on the plants its effects will be manifested when the plants are subjected to a temperature at which vegetative growth would be resumed.

A field experiment was undertaken on October 7 using *P. lanceolata* growing in Kentucky bluegrass sod. At that time several frosts and prevalent relatively low temperatures had checked the vegetative growth of both grass and plantain. The lawn area used was 70 feet square and contained a dense stand of plantain. It was equally divided into four smaller squares. An aqueous spray solution containing 1000 p.p.m. of the acid and 0.5% Carbowax 1500 was applied to two of the plots with a large power-sprayer at 400-lb. pressure at the rate of 5 gallons per 1000 sq. ft. The remaining two plots were untreated. Observations were made throughout the winter months, and on March 8, 1945, detailed counts of the living plantains found in ten randomized square-yard areas within each plot were obtained.

Although there was slight epinasty during October, many of the treated plants retained green center leaves throughout November and December. As the temperature slowly began to rise late in February, however, distinct differences between the treated and untreated plants of *Plantago* became apparent. After a few warm days in early March many of the plants were dead. On March 8, 152 days after treatment, the average (ten randomized square-yard areas) numbers of living plantains per square yard in the two spray-treated and the two control plots were as follows: treated plots 2.4 and 2.0, and control plots 47.2 and 30.3, or a decrease in population of 94.9 and 93.4%, respectively, as a result of treatment.

Another field experiment was conducted on *P. lanceolata*, using a plot of turf 128 sq. ft. that contained a dense and relatively uniform stand of plantain. On December 19 it was sprayed, using a knapsack type of hand-pressure sprayer, with a solution containing 1000 p.p.m. of the acid and 0.5% Carbowax 1500 (5 gallons per 1000 sq. ft.). At the time of application the ground was frozen to a depth of 2 inches and the plants were dormant. The leaves of the preceding season were still attached and remained so throughout the 8 succeeding weeks, although some of them had developed brown areas at their tips from slight frost injury. On January 5, 1945, a comparable plot on adjoining turf was sprayed with an aqueous solution containing 1000 p.p.m. of the sodium salt of the acid at the rate of 5 gallons per 1000 sq. ft. No Carbowax was used in this salt solution, but instead a small quantity of soap was added as a spreader.

At 2-week intervals after the treatments had been applied, twelve treated and twelve untreated plants were lifted, the adhering soil thawed out, and the

plants set in composted soil contained in 4-inch pots and placed in a greenhouse maintained at 65°.

There was no evidence of epinasty or rolling of the petioles of the leaves of the dormant plants in turf that had been treated in December with either the acid or its sodium salt, so long as the plants remained outdoors in the frozen ground. Within a few days after the treated plants were brought into the greenhouse they began to show epinastic responses. The control plants grew vegetatively following transplanting.

As the untreated plants developed new growth, the old leaves of the treated ones gradually showed more epinasty, followed by yellowing and ultimately death. As shown in figure 3, the treated plants had developed growth responses which were typical of others treated and grown in the field at moderate temperatures and reported earlier (**6**). Plants brought into the greenhouse after remaining outside at subfreezing temperature for 6 and 8 weeks following treatment, and being subjected repeatedly to ice and snow during this period, responded in a typical way to the growth-regulating substance coincident with the initiation of growth in the untreated plants brought into the greenhouse at the same time. The number of days required for death was significantly less among the treated plants brought into the greenhouse 6 and 8 weeks after treatment than among those brought in 2 weeks after treatment.

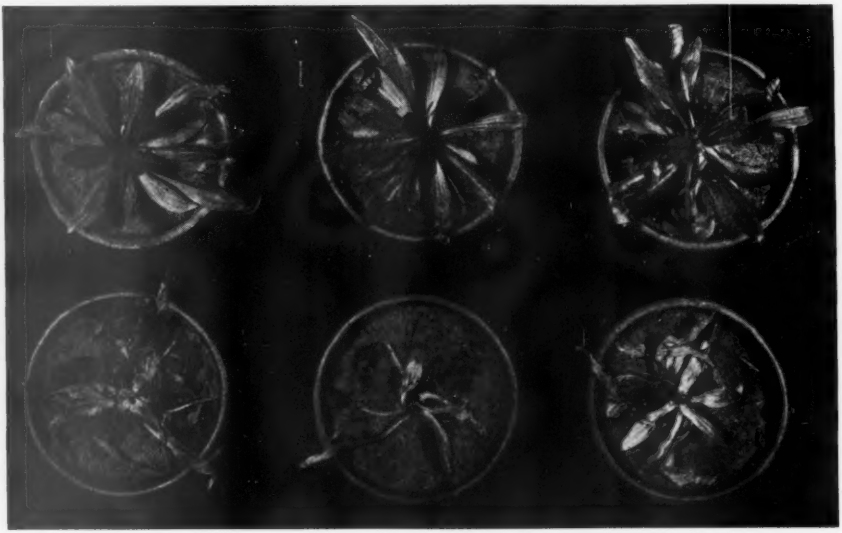

FIG. 3.—Killing of *Plantago lanceolata* by aqueous spray treatment with solution containing 1000-p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500 applied at rate of 5 gallons per 1000 sq. ft. in the field to plants in frozen ground. Sprays applied December 19 and plants moved to 65° greenhouse 17 days later. Photographed January 23, after 18 days in greenhouse. Upper row, unsprayed; lower row, sprayed.

OTHER SPECIES

In another experiment, 1-year old plants of *Plantago major* (broad-leaved

plantain) and of *Sisymbrium officinale* (hedge mustard) were transplanted from the field and grown for 1 week at 65°–75°, after which they were treated and maintained at this temperature. Included in the same experiment, plants of *Brassica juncea* (mustard) and *Convolvulus arvensis* (field bindweed) grown from seed planted in the greenhouse in late November were treated when 3–5 inches in height. For comparative purposes, *P. lanceolata* and *B. verna* were also included in this experiment. Spray treatment was with an aqueous solution of 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. containing 0.5% Carbowax 1500, and with the sodium salt of the acid at 1000-p.p.m. concentration.

Under greenhouse conditions, plants that were treated and grown at 65°–75° were all killed within periods of 10–21 days by either the sodium salt or the acid treatment (table 2). Under these conditions there was no significant difference between the salt and the acid with respect to their herbicidal activity. Both compounds when applied at 1000 p.p.m. to actively growing plants in the greenhouse resulted in killing of each of the six species investigated. The plants of the mustard family (*Barbarea*, *Brassica*, and *Sisymbrium*) appeared to be slightly more sensitive to the acid and were killed somewhat more quickly (2–5 days) than by the application of comparable amounts of the salt.

Rooted pieces of stems of *Stellaria media* (common chickweed) were planted in late November and of *Hydrocotyle rotundifolia* (lawn pennywort) and of *Prunella vulgaris* (heal-all) in the middle of December in flats (12″ × 24″). All flats were placed in a relatively cool greenhouse at 65°, and at the end of 45

TABLE 2

RELATIVE EFFECTIVENESS OF 2,4-DICHLOROPHENOXYACETIC ACID AND OF ITS SODIUM SALT APPLIED IN AQUEOUS SPRAYS (ON JANUARY 5, 1945) AT 1000-P.P.M. CONCENTRATION, AS INDICATED BY NUMBER OF DAYS REQUIRED FOR COMPLETE KILLING OF ALL PLANTS OF SIX SPECIES GROWING AT 50°–60° AND 75°–90° F. CARBOWAX 1500 AT CONCENTRATION OF 0.5% INCLUDED IN ACID SPRAYS; SMALL QUANTITY OF SOAP INCLUDED IN SALT SPRAYS NOT CONTAINING CARBOWAX. UNTREATED CONTROLS WERE NOT ADVERSELY AFFECTED BY THE TEMPERATURE CONDITIONS IMPOSED

SPECIES	NO. PLANTS PER TREATMENT	DAYS REQUIRED FOR KILLING OF ALL PLANTS			
		2,4-dichlorophenoxyacetic acid at 1000 p.p.m.		Sodium salt of acid at, 1000 p.p.m.	
		First 21 days at 50°–60°; remainder at 75°–90°	Continuously at 75°–90°	First 21 days at 50°–60°; remainder at 75°–90°	Continuously at 75°–90°
Barbarea verna	15	28	15	28	18
Brassica juncea	20	23	10	25	15
Convolvulus arvensis	16	.....	14	.....	14
Plantago lanceolata	12	31	21	31	21
Plantago major	10	31	18	28	18
Sisymbrium officinale	2	.....	10	.....	12

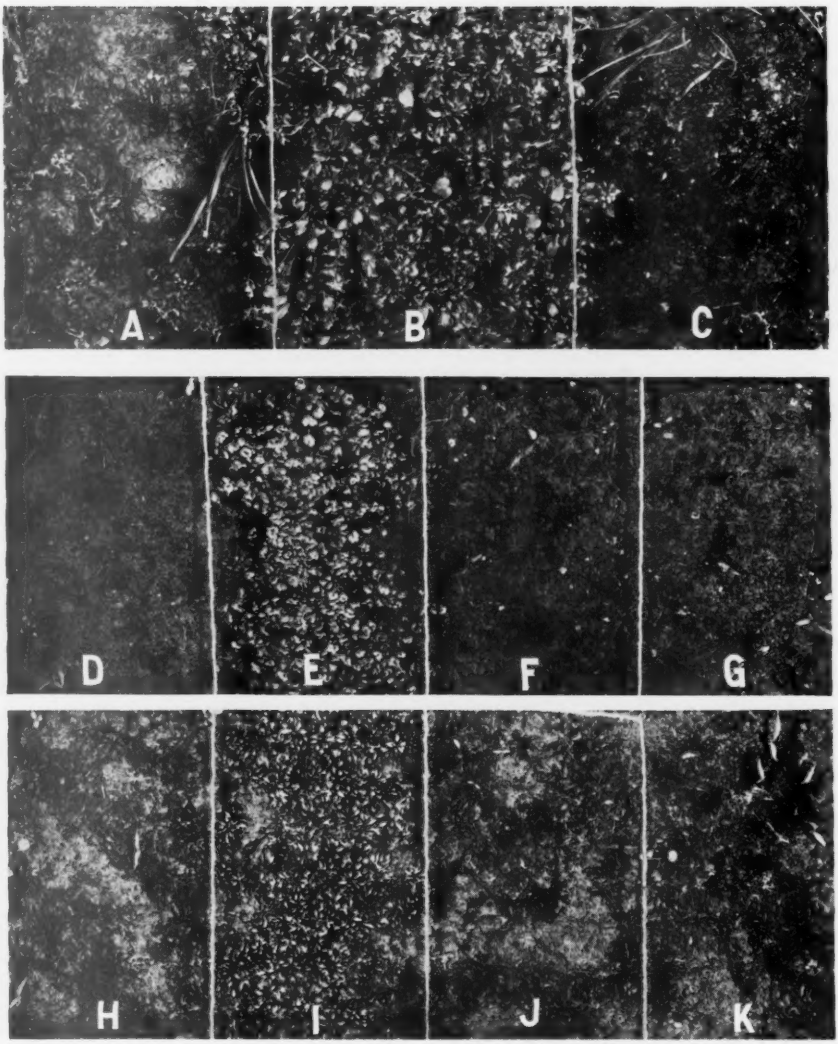

FIG. 4.—Effects of aqueous sprays containing 2,4-dichlorophenoxyacetic acid or its sodium or ammonium salt applied at 1000-p.p.m. concentration at rate of 5 gallons per 1000 sq. ft. to flats in the greenhouse. Upper row, *Stellaria media;* middle row, *Hydrocotyle rotundifolia;* lower row, *Prunella vulgaris*. *A*, sprayed, sodium salt; *B*, unsprayed; *C*, sprayed, acid; *D*, sprayed, sodium salt; *E*, unsprayed; *F*, sprayed, ammonium salt; *G*, sprayed, acid; *H*, sprayed, ammonium salt; *I*, unsprayed; *J*, sprayed, acid; *K*, sprayed, sodium salt. Carbowax 1500 at concentration of 0.5% used in the acid sprays; small quantity of soap included in the salt sprays, which were applied without addition of Carbowax.

days the plants were well established. Each of three flats of each species was divided into three or four sections for treatment. For comparative purposes, individual sections in each flat were treated with the acid while others received the sodium or ammonium salt, leaving one section in each flat untreated. A measured volume of aqueous spray was applied in each instance, so that treatment consisted of equivalent amounts of the various compounds at the rate of 5 gallons per 1000 sq. ft. of the spray at 1000-p.p.m. concentration. Carbowax 1500 was included in all of the acid sprays at 0.5% concentration, while the salt sprays were prepared with a small quantity of soap without the addition of the wax spreader.

At the time of treatment the *Stellaria* had made a rank vegetative growth 3–4 inches in height and completely covered the flats, while the plants of *Prunella* and *Hydrocotyle* were growing more slowly and had covered approximately 75–80% of the surface area. After treatment, the flats remained in the 65° greenhouse for a period of 10 days and then were shifted to a warm greenhouse at 75°–80°.

The effectiveness of the ammonium as well as of the sodium salt of the acid was found to be accelerated at increased temperature. This was true also when the compound was applied under the different temperature conditions in the acid form. The *Hydrocotyle* and *Prunella* in a 60° greenhouse failed to show the usual symptoms following treatment with the acid or with its sodium or ammonium salt over a period of 10 days. When the flats were moved to a higher temperature (75°–80°), however, the plants were killed within 4 days (fig. 4). In the same experiment, *Stellaria* that was growing luxuriantly was killed at 65° when treated with either the sodium salt or the acid.

Summary

1. Plants of *Barbarea verna* and *Plantago lanceolata* which were established in 4-inch clay pots in a warm greenhouse (65°–75° F.) were moved to greenhouses in which temperatures of 32°–40°, 50°–65°, 65°–75°, and 75°–90° F. were maintained, and separate lots of twenty-four plants each were spray-treated with 0, 500, 1000, and 1500 p.p.m. concentrations of 2,4-dichlorophenoxyacetic acid 1 week later. Carbowax 1500 was included in the acid sprays.

2. Treatment at the two higher temperature ranges resulted in rapid killing (18–21 days). All three concentrations of the acid were about equal in their effectiveness under the conditions of the experiments. At 50°–60°, however, the time required to kill plants with the acid treatment was extended for 11–15 days longer, while at 32°–40° the treated plants were still living at the end of 50 days following spray applications.

3. The data indicate that the acid was present in lethal amounts, either within the tissues or on the surface of plants, treated in the greenhouse and held at a relatively low temperature (32°–40°).

4. Spray treatments with 1000 p.p.m. concentration of the sodium salt of the acid were about equal to the acid in effectiveness in causing death of *Barbarea verna, Brassica juncea, Convolvulus arvensis, Plantago lanceolata, P. major,* and *Sisymbrium officinale.* The salt likewise was found to be present in or on plants treated at low temperature (50°–60°), since upon removal to a warmer temperature they died rather quickly.

5. The ammonium as well as the sodium salt of the acid was effective in killing *Hydrocotyle rotundifolia, Prunella vulgaris,* and *Stellaria media.* Death of plants of *Hydrocotyle* and *Prunella* was

hastened by removal from a greenhouse at 60° to one maintained at 75°–80°.

6. Field experiments have demonstrated that dormant *Plantago lanceolata* in frozen ground, having some living leaves, when treated with the acid or its sodium salt (applied in spray solution of 1000 p.p.m. at 5 gallons to 1000 sq. ft.) is killed when the plants are placed under temperature conditions favorable for growth.

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BELTSVILLE, MARYLAND

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EFFECT OF ALGASTATIC AGENTS ON MARCHANTIA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **568**

PAUL D. VOTH

Introduction

In recent years, certain of the aromatic diamidines have been utilized in the control of protozoan infections and in the control of bacteria in chronic wound sepsis. Propamidine and stilbamidine have received the greater share of attention, and their qualities, especially as they affect the physiology of human beings and of animals, have been described by ASHLEY *et al.* (1), DAUBNEY and HUDSON (4), DEVINE (5), FULTON and YORKE (6, 7), KIRK and HENRY (8), LOURIE and YORKE (10), THROWER and VALENTINE (12), and by many others. The trypanocidal action of pentamidine has been described by DAUBNEY and HUDSON (4) and by SAUNDERS *et al.* (11). Oxidation of nitrogenous compounds by *Escherichia coli* and other bacteria is inhibited by propamidine (3, 2, 9).

The effectiveness of the diamidines in killing or in retarding the growth of protozoa and bacteria suggested the possibility that these substances might arrest the growth of the common blue-green and green algae which infest ex-

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perimental cultures of *Marchantia* and other plants.

Relatively few species of algae found naturally in the greenhouses at the University of Chicago become a problem in the culture of *Marchantia*. Of the green algae, *Chlamydomonas* sp. becomes established on moist glass cloth within a few days, and *Stichococcus subtilis* (Kütz.) Klerck. is a common contaminant, rapidly developing into yellow-green, stringy masses.

At least two blue-green algae are pioneers in a greenhouse algal succession. *Nostoc muscorum* Born. & Flah. appears as bright blue patches on glass cloth, usually at the bases of recently transplanted tips of *Marchantia*. Another blue-appearing alga is the finely filamentous *Plectonema nostocorum* Gom., which coils into small irregular masses—as seen microscopically. Blue-green algae which appear in great abundance after 3–4 weeks are *Chroococcus rufescens* (Kütz.) Naeg., which is bright blue-green in young cultures but appears brown or black in older ones, and the filamentous, black, *Phormidium autumnale* Gom. The latter grows vigorously and not only surrounds but also covers entire plants of *Marchantia*, actually causing the death of experimental and of stock plants.

When stock cultures are permitted to grow undisturbed for several years with areas of glass cloth unoccupied by *Marchantia*, a climax in algal succession is reached. *Entophysalis cornuana* Sauv. and *Amphithrix janthina* Born. & Flah. constitute most of the $\frac{1}{2}$-inch algal layer. On soil cultures *Oscillatoria sancta* Gom. is common, but it is not a part of the glass-cloth flora.

The effects of copper salts, potassium permanganate, and the aromatic diamidines—propamidine, pentamidine, phenamidine, and stilbamidine—on the growth of algae and on the vigor of several clones of *Marchantia polymorpha* are reported here.

Material and methods

In preliminary experiments involving the use of copper salts and potassium permanganate, a variety of clones of *M. polymorpha* were tested. In the more extensive trial involving the diamidines, five clones were utilized.

Cultures C and D were male and female plants, respectively, isolated from the charcoal ashes of a camp fire in a second-growth forest near Ohiopyle, Pennsylvania, in June, 1940. These are characterized by a yellow-green color, many gemmae cups, and a dorsally visible midrib. This form of *M. polymorpha* is common from the Atlantic coast to the Black Hills of South Dakota.

Culture Pa (female) originated from plants growing along Stump Spring Road, Big Creek P.O., Huntington Lake, Fresno County, California, at an elevation of 5500 feet. These plants are characterized by a dark-green color, few gemmae cups, a prominent dark-green midrib, and purplish-red lower epidermis and appendages due to the presence of anthocyanin in the cell walls. This clone represents the aquatic form of *M. polymorpha* found locally from Michigan to California and Oregon.

Cultures Q and R (male and female, respectively) originally grew on moss mats and boulders along the water-soaked banks of Deer Creek, Lakeshore, Huntington Lake, Fresno County, California, at an altitude of 7000 feet. These clones grow slowly, usually are yellow-green in color, develop fewer gemmae cups than the eastern form, possess no dorsal midrib line, and are somewhat fleshy. They represent the western form

of *M. polymorpha*. This thallus type has been found in Florida, the Black Hills of South Dakota, Montana, and California. Clones Pa, Q, and R were collected by Dr. Charles H. Quibell in August, 1941.

Through the courtesy of Dr. D. F. Robertson, Merck and Company, four aromatic diamidines were made available. They are:

Propamidine.—4:4′-diamidino-1:3-diphenoxypropane dihydrochloride, $C_{17}H_{20}N_4O_2 \cdot 2HCl$

Pentamidine.—4:4′-diamidino-1:5-diphenoxypentane dihydrochloride, $C_{19}H_{24}N_4O_2 \cdot 2HCl$

Phenamidine.—4:4′-diamidinodiphenyl ether dihydrochloride, $C_{14}H_{14}N_4O \cdot 2HCl$

Stilbamidine.—4:4′-diamidinostilbene diisethionate, $C_{16}H_{16}N_4 \cdot 2C_2H_6O_4S$

Their chemistry has been discussed by ASHLEY *et al.* (1), WIEN (15), and LOURIE and YORKE (10). In the nutrient solution used, all four substances are soluble up to 100 p.p.m., except stilbamidine—which is soluble to about 10 p.p.m.

The culture method described previously (13) consisted of arranging six plant tips on a disk of glass cloth supported by a glass rack and placed in the open half of a large moist chamber. Plants were lightly rinsed with distilled water, after which the appropriate solution was added. Per liter, the stock nutrient solution contained 1.6 ml. KNO_3, 1.4 ml. $Ca(NO_3)_2$, 1.2 ml. $Mg(NO_3)_2$, 0.8 ml. KH_2PO_4, and 1.6 ml. $MgSO_4$—each in 0.5*M* concentration (14). Micronutrients $MnSO_4$, $ZnCl_2$, and $Na_2B_4O_7$ were present in 0.2-p.p.m. and $FeSO_4$ in 0.02-p.p.m. concentrations.

After 12 days of growth on plain nutrient solution, certain cultures were supplied with 100, 10, or 1 p.p.m. of each of these compounds. Control cultures were also maintained. Owing to the severity of the 100-p.p.m. treatment, all such cultures were harvested 22–23 days after planting. Plants on lesser concentrations were grown 28–30 days. For the principal experiment here reported, no. 19, the natural photoperiod of October prevailed.

When one of the six plants in a culture was preserved in formalin-acetic acid-alcohol, fresh weights of the six plants—and also of the remaining five—were recorded, as well as gemmae-cup numbers and the area (determined photometrically). When one plant was preserved, the dry weight of six plants was calculated on the basis of the percentage dry weight of five plants and the fresh weight of six.

Longitudinal and cross-sections were cut at 7–10 μ from sample plants imbedded in paraffin and stained with safranin, gentian violet, and orange G.

Investigation

METALLIC DERIVATIVES

COPPER SALTS.—In February and March, 1943, cupric acetate ($Cu[C_2H_3O_2]_2 \cdot H_2O$) or cupric tartrate ($CuC_4H_4O_6$) was added to the nutrient solution supplied to alga-infested cultures of *Marchantia*. Concentrations of 20, 2, 1, 0.8, and 0.4 p.p.m. were used. Each day the solutions were discarded, the plants and glass cloth rinsed, and new solution containing the specific copper salt added.

In the cultures receiving 20 p.p.m. of either copper salt, all plants of *Marchantia* became dark green with tan borders, and they died after 4 days. When exposed to direct sunlight for a day, the liverworts bleached to a light tan. All algae also bleached, indicating that they too had died. Treatments with 2 p.p.m. reduced the algal population but apparently did not injure the *Marchantia*.

Lower concentrations produced no observable effects. After a week, treatments containing 2 p.p.m. proved to be injurious, but the 1-p.p.m. treatments seemed to loosen the algae from the glass cloth without injury to the liverwort. Eight daily treatments at 1 p.p.m. sufficed to kill all algae in several cultures. Concentrations of 0.8 and 0.4 p.p.m. appeared to inhibit algal growth for a few days, but after a week the glass cloths again became bright green with a fresh algae cover.

In the case of copper acetate, further daily changes of nutrient solution containing 1 p.p.m. resulted in renewed growth of both *Marchantia* and algae. The same concentration of copper tartrate acted differentially, however, in that it suppressed algae and permitted development of the liverwort.

On the basis of these results, most stock cultures were subsequently supplied with 1 p.p.m. of copper tartrate, but during the warm sunny days of early spring the basal portions of most plants of *Marchantia* became tanned, indicating toxic effects of the copper salt. Despite careful rinsing with distilled water and renewal of the nutrient solution without copper salts, all the plants in many cultures died. It is not definitely known whether in *Marchantia* the copper salts are excessively toxic or whether their action is cumulative.

In recent months the effectiveness of cupric sulphate ($CuSO_4$) as a suppressor of algal growth was determined by employing nutrient solutions containing 1590, 159, and 16 p.p.m. of this salt. All plants of *Marchantia* died within 3 days when the greatest concentration was applied; with 159 p.p.m., necrotic zones appeared on the margins. Both treatments effectively killed all algae. After 21 days of treatment with 16 p.p.m., no severe injury was evident in the liverwort but all thalli were roughly one-third smaller than in the control plants. Since some algae still persisted at this concentration of copper sulphate, further tests employing copper salts were abandoned.

The watery appearance of *Marchantia* when treated with copper salts at concentrations as low as 1 p.p.m. and the tendency of such salts to kill whole cultures of the liverwort resulted in abandoning the treatments with copper salts, and tests with other substances were initiated.

POTASSIUM PERMANGANATE.—Saturated solutions of this salt were prepared in 500-ml. lots in the moist-chamber halves serving as culture dishes. The entire culture of *Marchantia* attached to the glass cloth was submerged in the saturated permanganate solution for about 5 minutes. The cultures were then removed from the solution, and, after the tips of the plants had been sprayed lightly with distilled water, the excess solution—still colored—was drained off. Washing was continued with tap or distilled water until the drainage water was colorless. Many of the algae adhering to the glass cloth had become a rich brown in color and could be sprayed off rather readily with a stream of water. Older parts of the *Marchantia* thallus became brown or even black, but the growing cells remained alive within a radius of 2–3 mm. of the apical notch—even when treatment had been exceptionally rigorous. For a few days following treatment, especially when high greenhouse temperatures prevailed, growth was negligible. After this initial period of retarded growth, the plants branched and grew rapidly. In stock cultures, where as many as 100 plants are well attached to the glass cloth, the entire cloth surface (except the margins) is soon covered with

the liverwort, thus retarding and preventing the re-establishment of algae.

Although the potassium-permanganate treatment is severe and time-consuming, it is useful in producing robust, algae-free stock plants for experimental use. It would, however, greatly complicate possible interpretation of results of experiments dealing with the effects of variation in nutrient supply on growth.

MANGANESE SULPHATE.—A few clones of *Marchantia* were treated with 1600, 160, and 17 p.p.m. of manganous sulphate ($MnSO_4 \cdot H_2O$). The highest concentration killed the liverwort within a week; 160 p.p.m. resulted in much-stunted thalli; and the lowest concentration effected only slight reduction in the width and length of the plants. Algal growth was completely suppressed by the higher concentrations of this sulphate. The 16-p.p.m. concentration effectively reduced the total area of *Marchantia*, but many algae were able to establish themselves on the glass cloth. Since manganous sulphate was ineffective as an algastatic agent, its use was discontinued.

AROMATIC DIAMIDINES

Each of 100 culture dishes, equipped with glass racks and glass cloths, was planted with six tips of *Marchantia* from the appropriate clone. A transplant was 1.1 sq. cm. in area, in the form of an acute triangle with a base of 1 cm. cutting the thallus transversely about 1 mm. in front of the apical notch. The plant fragments were cut with razor blades held in a metal instrument, thus attaining uniformity in size of apical transplant pieces and increasing the speed of planting. All growing points except one were removed from each transplant to insure a still greater degree of uniformity. A few dishes contained an extra plant, which was preserved in F.A.A. killing fluid at harvest time and subsequently used for anatomical study. All cultures were supplied with the stock nutrient solution for 12 days.

Ten-milligram lots of each of the diamidines—propamidine, pentamidine, phenamidine, and stilbamidine—were weighed out daily and dissolved in 1 liter of nutrient solution. This constituted the 100-p.p.m. solution for each substance. One hundred milliliters of this concentrated solution was diluted with 900 ml. of nutrient to yield a solution with a concentration of 10 p.p.m. Using 100 ml. of the latter solution, another dilution was prepared containing 1 p.p.m. of the respective diamidine. Beginning with the thirteenth day after planting, each culture of six *Marchantia* plants was rinsed with a spray of distilled water, the old nutrient solution discarded, and about 115 cc. of the proper solution added daily. All dishes were compactly arranged on a large table and rotated in a regular manner every third day to minimize differences in light intensities and in evaporation rates in marginal and central dishes.

After 1 week, plants receiving 100 p.p.m. pentamidine began to show marginal necrosis. Clone Q was affected earliest and most severely. At harvest time, plants of this clone were roughly ovate in shape, with much-constricted terminal growing lobes. The broadest portion had developed before treatment with pentamidine began. Hence in Q, approximately only 15% of the thallus area was formed in the 11 days of pentamidine treatment. The gross appearance of all clones after treatment with the diamidines is summarized in table 1.

As judged by the color of the glass cloths, only two substances—propamidine and pentamidine—suppressed all

algal growth when applied in concentrations of 100 p.p.m. In treatments with phenamidine or stilbamidine, in the same concentration nearly every glass cloth became at least slightly green, indicating the presence of green algae and possibly of *Chroococcus*. As already noted, pentamidine not only suppresses algal growth but also causes the margins of *Marchantia* thalli to die and turn tan in color. Thus propamidine, which is able to reduce algal growth to a minimum and yet not cause local or general necrosis of *Marchantia*, gives the greatest promise of being a desirable algastatic compound.

Effects of the four diamidines on area and dry weight of *Marchantia* are presented in table 2.

Plants in cultures having a concentration of 100 p.p.m. of propamidine, pentamidine, or phenamidine attained about one-half (or in some instances about one-fourth) the size of control plants. As is to be expected, the dry weight of these plants is also much less than that of the control plants—as little as one-half or only one-third. In contrast to the other diamidines, with stilbamidine the area and dry weight are greater. While this characteristic seems to be desirable, it will be shown subsequently that lower concentrations of this material are less effective in controlling the growth of algae than are propamidine and pentamidine.

Gemmae cups tend to appear in greater numbers on male (C, Q) than on female plants (D, R), as reported for other clones in previous experiments (**13**, **14**). Clone Pa, which originally grew in a semi-aquatic habitat, rarely produces gemmae cups, even when the proportions of anions and cations are varied.

TABLE 1

GROSS APPEARANCE OF MARCHANTIA CLONES GROWN ON 100 P.P.M. OF THE SUBSTANCES FOR 11 DAYS

Treatment	Approximate area alive or green (%)	Algae	Type of necrosis	Additional observations
Propamidine	25	None	Basal and general	Color of dry oak leaves
Pentamidine	75 in C, 25 in others	None	Marginal in Pa, Q, R	Dark tan color
Phenamidine	10 in D, 75 in others	Few	Small spots (nearly total in D)	Black tips or black spots near notch in C, Pa, Q
Stilbamidine	95	Few	None except in D	Color bright green (D pale green)
Control	100	Usually many	No necrosis	Tips of some Pa plants watery

Marchantia plants growing on 10- and 1-p.p.m. concentrations of the diamidines were permitted to grow an additional week before being harvested, so that each culture received twenty doses of the respective diamidine. Dry weights of clones C, D, Q, and R are plotted in figures 1–4. Two cultures of six plants each of the four clones were grown on plain nutrient solution as the respective controls for all four treatments.

The effects of propamidine, pentamidine, and phenamidine on the growth of *Marchantia* are strikingly similar (figs.

TABLE 2

EFFECT OF ADDING 100 P.P.M. OF A DIAMIDINE TO NUTRIENT SOLUTION SUPPLIED TO SIX PLANTS OF MARCHANTIA POLYMORPHA. FOR CLONES C, PA, AND Q, TWO LOTS OF SIX PLANTS EACH WERE AVERAGED

Treatment	Area (sq. cm.)	Dry weight (cgm.)	No. of gemmae cups
	Clone C♂		
Propamidine	21.0	8.0*	13
Pentamidine	26.5	8.4*	10.5
Phenamidine	22.5	11.4*	14.5
Stilbamidine	35.0	14.4*	24
Control	55.5	20.5*	35
	Clone D♀		
Propamidine	26.0	7.3	14
Pentamidine	27.0	8.6	7
Phenamidine	22.0	8.7	9
Stilbamidine	28.0	10.8	12
Control	77.0	22.0*	45.5
	Clone Pa♀		
Propamidine	27.0	8.5	1
Pentamidine	28.0	9.6	0
Phenamidine	30.5	12.8*	0
Stilbamidine	34.5	13.2	0
Control	80.0	19.2	0
	Clone Q♂		
Propamidine	23.0	9.2*	7.5
Pentamidine	24.5	10.2*	6
Phenamidine	21.5	12.0*	10
Stilbamidine	33.0	14.4*	22.5
Control	41.0	17.4*	21.5
	Clone R♀		
Propamidine	23.0	9.9	3
Pentamidine	25.0	9.9	2
Phenamidine	30.0	13.6	4
Stilbamidine	31.0	14.3	10
Control	44.0	16.7	11

* Calculated dry weight (at least in part) based on five plants.

1–3). With the exception of the dry weight of clone R grown on 10 p.p.m. propamidine, clones C, D, and R show a relatively low weight on 10 p.p.m., a high weight on 1 p.p.m., and an intermediate weight on the control.

At the time of harvest, the distribution and relative abundance of the algae in cultures treated with the lower concentrations of the diamidines and in the control were noted. Some algae appeared in all cultures. Only a faint green color was apparent at the margins of glass cloths in cultures treated with 10 p.p.m. propamidine and 10 p.p.m. pentamidine. In the latter treatment the basal margins of the plants were dry, again indicating the tendency of this compound to kill peripheral areas of the liverwort. Glass cloths in cultures receiving 10 p.p.m. phenamidine were uniformly covered with a conspicuous layer of green and blue-green algae. One culture of D treated with 10 p.p.m. stilbamidine developed no algae, but all others similarly treated supported an abundant algal cover.

Cultures treated with 1 p.p.m. of the four diamidines all supported some algae. Propamidine, pentamidine, and phenamidine cultures were about equally effective in controlling growth of the green algae. Cultures Q and R treated with either of these three substances were rather heavily infested with blue-green algae, especially *Phormidium* and *Chroococcus*. Nevertheless, the dry weight of R in each instance was greater than the weight of this clone when grown on the control solution. Cultures of C and D treated with phenamidine and stilbamidine in 1-p.p.m. concentration also were infested with *Phormidium* and other blue-green algae. The algastatic action of these compounds

in this minute concentration permitted a marked increase in dry weight, suggesting that the optimum benefit to be derived from these substances is near 1 p.p.m.

Control cultures were all heavily infested with all algae encountered in this investigation.

Clone Q appears to be a special problem, since it reacts unfavorably to all four diamidines with striking uniformity. Additional tests are desirable to determine whether this behavior is characteristic at all seasons of the year. It is possible that *Phormidium autumnale* and similar algae were growing with the central strand of pegged rhizoids and thus were in a favorable position to contaminate all cultures with a vigorous mass of trichomes. Plants of this clone growing in one dish and treated with 1 p.p.m. stilbamidine developed an almost pure culture of a diatom, *Nitzschia communis* var. *obtusa* Grun., which covered the glass cloth completely except for two small raised areas. Dr. Ruth Patrick kindly identified this plant.

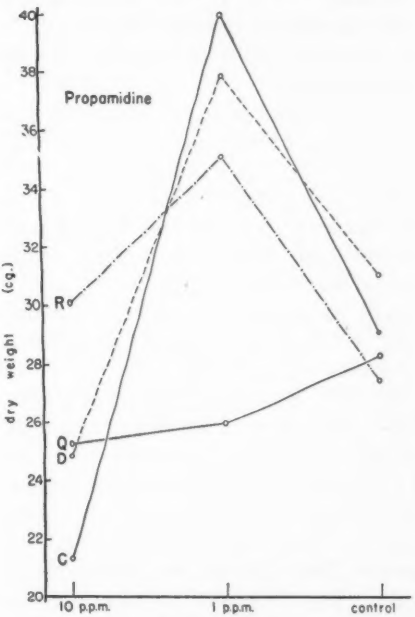

FIG. 1.—Dry weights of four clones of *Marchantia* grown on 10 and 1 p.p.m. propamidine and on untreated nutrient solution. Weights of clones D and Q (10 p.p.m.), C and Q (1 p.p.m.), and all control plants are based on two cultures of six plants each; other weights by one culture.

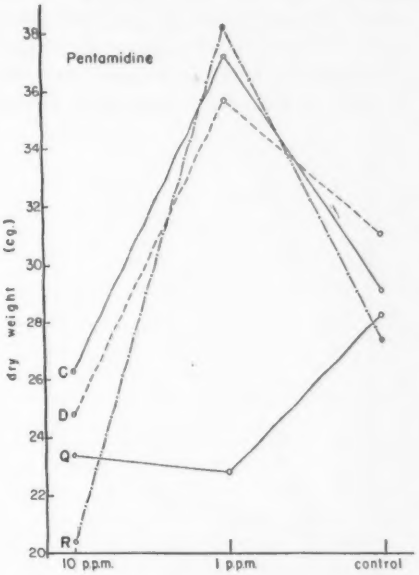

FIG. 2.—Dry weights of plants of *Marchantia* grown on dilute solution of pentamidine and on untreated nutrient solution. Weights of clones D and R (10 p.p.m.), C and Q (1 p.p.m.), and all control plants are based on two cultures of six plants each; others on one culture.

Stilbamidine reacted somewhat differently from the other diamidines. In 100-p.p.m. concentration it depressed the growth of *Marchantia* less than the other compounds. In concentrations of 10 and 1 p.p.m. the effects of this substance are somewhat variable, as is shown in figure 4. As plotted, in all three treatments

clone C was infested with *Phormidium* and other algae, thus accounting for its low dry weights. In contrast, the cultures of clone D had developed only a few algae, mainly green, permitting an unusually vigorous development of its plants. Clones Q and R followed their respective patterns of response, as judged by treatments with other diamidines. On the basis of the results recorded in tables

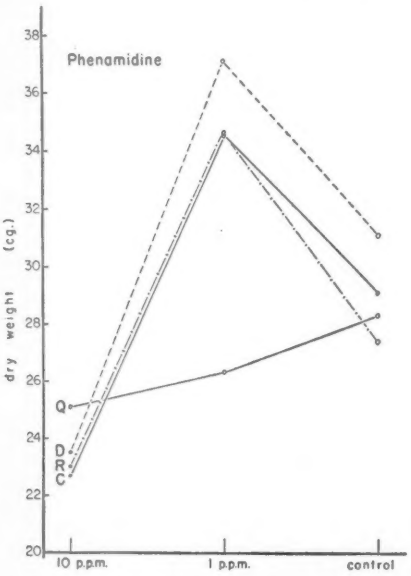

FIG. 3.—Dry weights of plants of *Marchantia* grown on dilute solutions of phenamidine and on untreated nutrient solution. Weights on same basis as in fig. 2.

1 and 2 and in figure 4, stilbamidine is not an effective algastatic compound.

To test the ability of the diamidines to kill algae or to suppress their growth, two series of cotton-plugged 15-cm. test tubes were prepared. Some tubes contained 10 ml. of control nutrient solution; others contained 1-, 0.1-, or 0.01-p.p.m. concentrations of each of the four diamidines. Each test tube of one series was inoculated with several drops of a dark-green suspension consisting mainly of *Chlamydomonas*. In the second series no inoculations were made; instead, nutrient solution which had been exposed to greenhouse conditions for several hours was used in making up the various concentrations of diamidines. After one month (November, 1944), the relative turbidity of the solutions was noted. In the inoculated series of test tubes no conspicuous or consistent differences in turbidity and green color were discernible. Control cultures, as well as those containing 10–0.01 p.p.m. of one of the four diamidines, ranged from 50 to 100% in turbidity, using the greatest density as a standard.

In the series prepared without inoculation, four control tubes and those containing 0.01 p.p.m. propamidine, 0.01 p.p.m. pentamidine, 0.1 and 0.01 p.p.m. phenamidine, or 0.1 and 0.01 p.p.m. stilbamidine were 50% turbid. Tubes containing concentrations of 10 and 1 p.p.m. of propamidine and pentamidine and 10 p.p.m. of phenamidine and stilbamidine were entirely devoid of green color. The remaining tubes, containing lesser amounts of the diamidines, were slightly turbid. From these trials on *Chlamydomonas*, and from extensive use of the diamidines in the culture of stock plants of *Marchantia*, it is obvious that none of these substances is able to eliminate a copious growth of algae from a culture at concentrations which are not also detrimental to the liverwort. When algae are few in number, a culture of *Marchantia* may be kept relatively free by applications of 1 p.p.m. of propamidine or pentamidine.

To determine the possible effect of propamidine on initiation and growth of rhizoids, gemmae of several clones representing the eastern, western, and aquatic forms of *Marchantia* were planted in agar

plates. Rhizoids formed as readily and as extensively in treatments at 100, 10, and 1 p.p.m. as in control plates. After about a week, gemmalings receiving 100 p.p.m. propamidine were stunted, their margins upturned, and their color a dull, translucent green. Propamidine does not therefore affect the development of rhizoids but instead affects the aerial portions, especially the margins of the thallus, where photosynthetic tissue is predominant.

Of special interest is the absence of tumors, regenerated areas, and other malformations in the thallus of *Marchantia* when treated with diamidines in concentrations of 100 p.p.m. or less. Approximately 150 microscopic slides were prepared of longitudinal and cross-sections of the forms of *Marchantia* used under every experimental condition here reported. The drying of the older parts of the thallus and the margins in treatments with higher concentrations of pentamidine seems to be no different histologically from the drying of the exposed margins of the control plants. No conspicuous reduction in cell size was discernible, suggesting that the narrower thalli occurring in the 100-p.p.m. treatments may be due to fewer cell divisions in the meristematic region of the apical notch. The shorter thalli probably result from fewer divisions of the apical cell.

Discussion

Additional tests are desirable to determine the specific relationships between low concentrations of the diamidines and the ability of plants similar to *Marchantia* to utilize fully anions (such as nitrates, phosphates, and sulphates), and also the cations, commonly supplied to plants. WIEN (15) reported that additional calcium decreases the fall in blood-pressure in animals treated with the diamidines here discussed. No information of any direct physiological effects on the cytoplasm in *Marchantia* is available. The constriction of the thallus of *Marchantia* treated with 100 p.p.m. of pentamidine resembles the narrowing of plants grown on solutions high in calcium and lacking in potassium and magnesium (13).

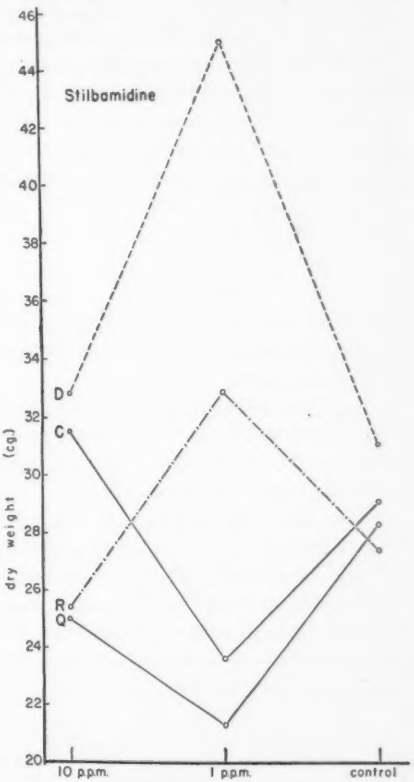

FIG. 4.—Dry weights of plants of *Marchantia* grown on dilute solutions of stilbamidine and on untreated nutrient solution. Weights of clones D and R (10 p.p.m.), C and R (1 p.p.m.), and all control plants are based on two cultures of six plants each; others on one culture.

Stilbamidine solutions subsequent to exposure to light for even a few hours are known to be toxic to man and animals (7, 8). No such toxic effects were evident

in the present experiments. Even though freshly prepared each day, the solutions were in open dishes and exposed to sunlight under greenhouse conditions. From the results reported here, stilbamidine is less toxic to algae and *Marchantia* than are the other three diamidines.

If in further tests a 1- or 2-p.p.m. concentration of propamidine interferes less with the growth of *Marchantia* than with interfering algae, investigations should be extended to determine the effect of this and other diamidines on the growth of moss protonemata and fern prothallia. An algastatic compound for use in cultures would be of value.

Summary

1. Green and blue-green algae which contaminate greenhouse cultures of *Marchantia* are named.

2. Copper acetate, tartrate, and sulphate are not sufficiently differential in their action, killing *Marchantia* as well as the algae they are to control.

3. Potassium permanganate in a saturated solution is useful in killing algae in stock cultures. Since all older portions of the *Marchantia* thallus are also killed, this treatment is not recommended for the control of algae in experiments involving mineral nutrition.

4. Propamidine, pentamidine, phenamidine, and stilbamidine stunt the growth of *Marchantia* and suppress algal growth at 100 p.p.m. At 10 p.p.m. most clones of *Marchantia* produce as much dry weight as the control plants. Algal growth is slight on treatments with propamidine and pentamidine; extensive on treatments with the other two diamidines. Treatments with 1 p.p.m. result in the production of much more dry weight in *Marchantia*, while many algae appear in small amounts in the cultures.

5. Propamidine interferes least with the growth of *Marchantia* and is as effective an algastatic agent as the other diamidines. Pentamidine produces marginal necrosis on *Marchantia* in the higher concentrations. Stilbamidine is the least effective of the diamidines tested.

6. Clone Q, a western form of *Marchantia*, failed to benefit from treatments with the diamidines.

The assistance of Mr. ROBERT BANDURSKI in many of the experiments is gratefully acknowledged. The algae were identified through the co-operation of Dr. FRANCIS DROUET of the Chicago Natural History Museum.

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CYTOHISTOLOGY OF THE REACTION OF MAIZE SEEDLINGS TO COLCHICINE[1]

JOHN E. SASS AND JOHN M. GREEN

Introduction

The production of autotetraploid plants by means of colchicine has become an established technique in plant breeding (1–5, 8). The doubling of the chromosome number is known to be the result of an inhibition of the anaphase mechanism, followed by the incorporation of a polyploid chromosome complement in a restitution nucleus (4). The specific action of colchicine on organ primordia, developing organs, tissues, and tissue systems is in need of further study in diverse taxonomic categories.

Relatively meager success has been achieved in attempts to double the chromosome number of maize with colchicine. The present study was undertaken to determine some cytohistological features of the responses of maize seedlings to colchicine in relation to the erratic production of tetraploidy.

[1] Contribution from the Iowa Agricultural Experiment Station, Ames, Iowa, in co-operation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U.S. Department of Agriculture. Journal Paper no. J. 1272 of the Iowa Agricultural Experiment Station, Project 182.

Material and methods

Iowa B1, an inbred line of yellow dent maize, was used for this study. Treatment of the seedlings was started when the third leaf was partially unrolled. The method used has been described by RANDOLPH (7) and consists of immersing the cut end of the radicle in a 0.05% aqueous solution of colchicine. The treatment may be continuous or may be alternated with immersion of the roots in water. The two methods have yielded similar results. The selected plants were subjected to continuous treatment for 91 hours, terminating when a majority of the seedlings were visibly swollen in the region of the coleoptile node (fig. 1). The identification of tetraploid plants in the field was based on pollen size and progeny tests.

Microscopic studies were made by the use of microtome sections of root tips and stem tips. The excised tissues were killed in a chrome-acetic-formalin solution, dehydrated with a dioxan series and imbedded in paraffin. Root-tip sections were stained in gentian violet-iodine or iron-haematoxylin. Stem-tip sections

were stained in iron-haematoxylin for cytological details and in safranin-fast green for histological features.

Morphological and cytohistological abnormalities

Maize seedlings treated by the foregoing method show three gross morphological abnormalities: stunting of the entire plant, enlargement of the nodes (fig. 1), and enlargement of the root tips. Treated seedlings have high mortality, and the survivors recover slowly.

FIG. 1.—Seedlings of maize: plants at left treated with colchicine, normal check plants at right

A low incidence of tetraploid sectors was found in the floral parts of treated plants. Pollen samples from approximately 200 plants were examined in 1943. Thirty-six of these plants yielded abnormally large, apparently 2n pollen. The average diameter of a sample of this large pollen was $127 \pm 1.87\ \mu$, whereas a sample from a diploid sib had an average diameter of $96 \pm 0.68\ \mu$ (fig. 2), corroborating the results obtained by RANDOLPH (6). Three of the progenies grown from selfed seed of these thirty-six plants consisted of tetraploids. The fact that the remaining progenies were apparently normal diploids is interpreted as evidence of sectoring in the tassel and possibly in the ear. RANDOLPH (6) has reported that the pollen of tetraploid

plants does not compete favorably with the pollen of diploid plants; therefore, in a pollen mixture from a tassel containing 4n sectors, pollination would be effected by the monoploid pollen.

A prominent histological feature of the thickened root tips of treated plants is the small size of the root cap (fig. 3). Mitosis and excessive cell expansion occur throughout the promeristem, producing the greatly thickened condition of the root tips, whereas the root cap remains very small, apparently as the result of inhibited mitosis and limited cell expansion.

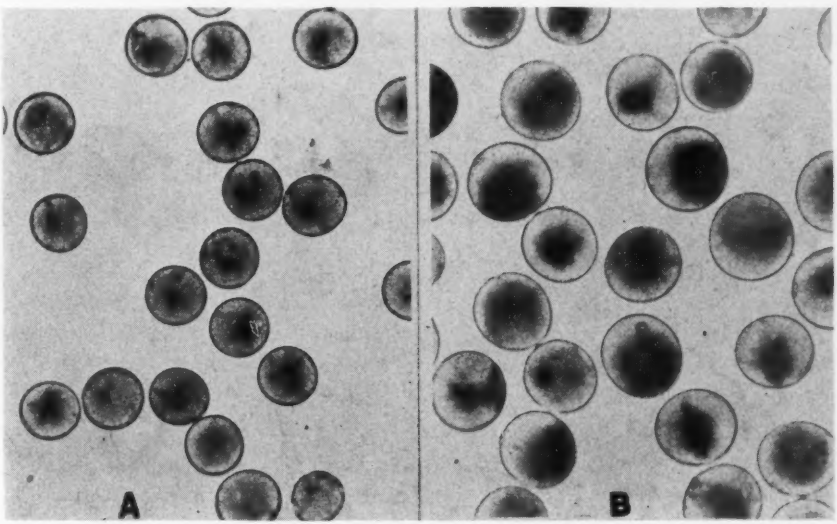
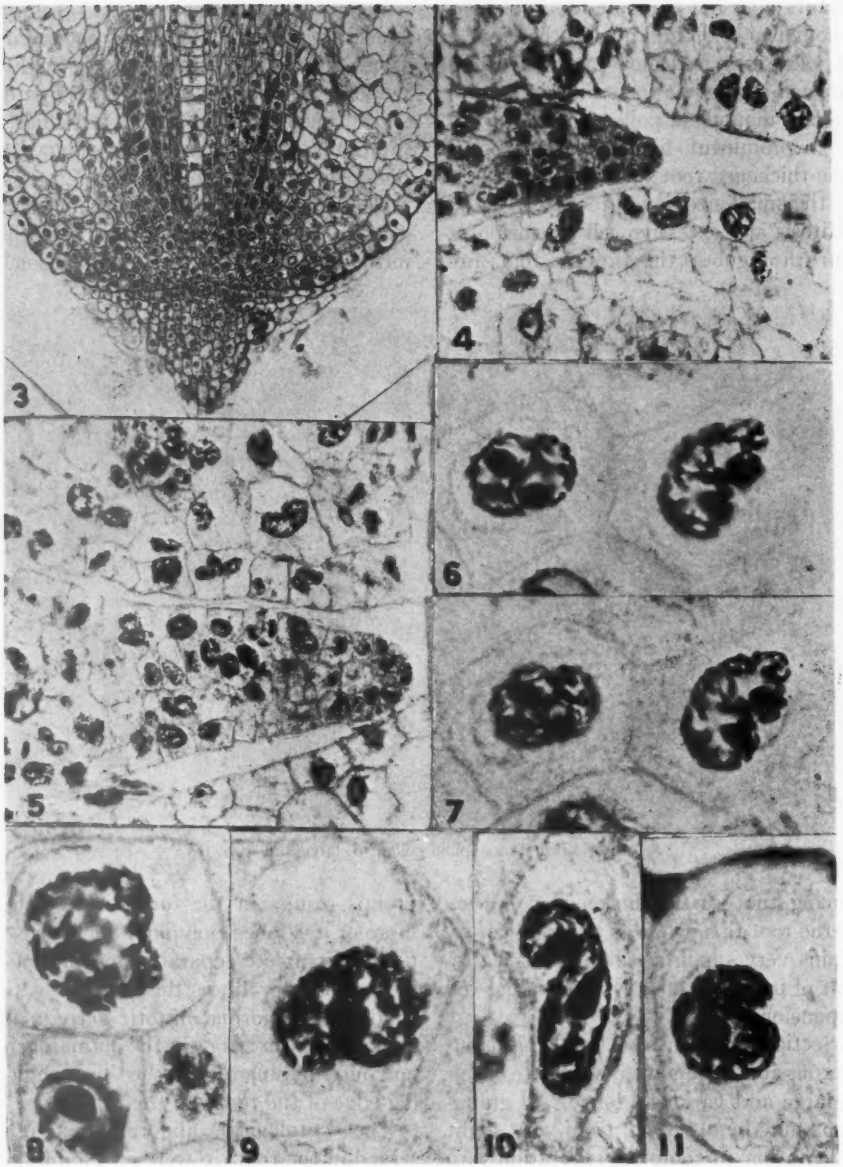


FIG. 2.—*A*, normal monoploid pollen; *B*, large diploid pollen

Sections of such root tips exhibit numerous abnormal nuclei, which are usually large and variously lobed. All gradations may be observed, from very slight indentation to complete separation into two or more nuclei (figs. 6–11). When these large nuclei are in late prophase (fig. 8), the chromosomes are sufficiently distinct to permit estimates of chromosome number, which show that the giant nuclei are polyploid. Chromosome counts from metaphase figures afford further evidence of polyploidy. Anaphase figures show much irregularity, particularly multipolar spindles, irregular separation of chromosomes into unequal groups, and excessive clumping of chromosome groups. Telophase reconstruction of the incompletely separated chromosome groups results in the formation of the more or less lobed polyploid nuclei (figs. 6–11). Complete separation of chromosome groups produces the multinucleate condition. Abnormal mitotic activity is most evident in a zone of the dermatogen and outer periblem, near the thin sloughing edge of the root cap (fig. 3).

The cytological abnormalities observed in the root tip were also found in the plumule. Sections of the promeristem of the stem of the seedling show relatively few abnormal nuclei, and the stem tip is a smooth dome. On the other hand,



FIGS. 3–11.—Fig. 3, longisection of root tip of treated plant, showing small root cap on swollen root. Fig. 4, transection of apparently normal marginal meristem of a leaf and affected surface cells of adjacent leaf tissues. Fig. 5, thickened marginal meristem and adjacent leaves, both showing nuclear abnormalities. Figs. 6–11, abnormal polyploid nuclei of treated plants.

the small, post-embryonic leaf primordia exhibit a striking response. The surface and subsurface cells become greatly enlarged, producing considerable thickening and a highly spongy cellular texture in the margin of the leaf primordium, especially on the distal edge (fig. 12). These leaf primordia exhibit to a marked degree the nuclear abnormalities previously described.

Aberrant mitosis also occurs in localized regions of the seminal leaves, which, except for the meristematic margins, are relatively more differentiated than the newly formed leaves. Abnormal mitosis occurs in the epidermis (fig. 4) and in the mesophyll (fig. 5). In figure 4 the marginal meristem of a leaf appears to have the normal thin edge, and the cells and nuclei are of normal size, whereas the older tissues of the adjacent leaf show a zone of expanded cells and giant polyploid nuclei. The marginal meristem in figure 5 obviously is abnormally thickened and shows nuclear abnormalities.

This study of the reactions of meristematic organs and histogens has shown some specificity of response. Inhibition of meristematic activity and of cell expansion in the calyptrogen contrasts sharply with the continued, though abnormal, mitotic activity in the adjacent promeristem and with the marked lateral cell expansion in the promeristem, plerome, and periblem. The latter condition is in accord with the observations of HAWKES (4), who ascribed transverse expansion and lack of extensive linear elongation of cells to a loss of cell polarity.

The erratic localization of the formation of tetraploid cells, particularly in the edges and thin portions of young leaves, in contrast with the scarcity of tetraploid cells in the massive promeristem, suggests that the tassel primordium as a whole is unlikely to be affected. It is more probable that the thin floral primordia are affected, resulting in the known production of tetraploid branches of the tassel. Cytohistological verification must await the study of field-grown plants during the period of inflorescence, floret, and floret-organ initiation.

FIG. 12.—Stem tip and post-embryonic leaf of treated plant, showing swollen leaf tip and abnormal nuclei (semidiagrammatic).

Summary

1. Seedlings of maize were treated with colchicine. Morphological abnormalities consisted of stunting of the seedling and swelling of the coleoptile node and of the root tip.

2. Sections of the root tip and plumule exhibit parallel mitotic aberrations—for example, multipolar spindles, clumping of chromosomes, multinucleate cells, and giant polyploid nuclei having varying degrees of lobing. These responses are

especially prominent in the marginal meristems of leaf primordia, the epidermis and mesophyll of young leaves, and in both the plerome and periblem of root tips.

3. The massive promeristem of the plumule is relatively unresponsive; cell expansion in the calyptrogen appears to be inhibited.

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CURRENT LITERATURE

Problems in Tree Nutrition. By M. C. RAYNER and W. NEILSON-JONES. London: Faber & Faber, Ltd., 1944. Pp. 184. 12/6.

This book presents detailed experiments on the use of various substances to enhance the value for the production of coniferous trees of certain British soils at Wareham Forest, Dorset. The correlation of growth and development of some species of conifers and oaks with the presence or absence of mycorrhiza, and the inoculation of soils with composts containing certain fungi, are discussed.

In the particular soils experimented upon, the presence of substances actively inimical to growth was demonstrated and the origin of the resulting toxicity confirmed. The toxins operate directly by inhibiting fungal growth and by almost complete cessation of cellulose decomposition. The indirect effects of such biological inertia are the restriction of root growth of trees, impeding of mycorrhiza formation, and curtailment of supply of nutritive requirements.

Justification for the use of organic composts for relieving infertility under field conditions has been provided by laboratory experiments proving that removal of toxicity and profound alteration of the organic substrate and soil bionomics follow addition of compost. Changes so induced are self-propagating and the effects on growth persistent.—E. J. KRAUS.

Hayfever Plants. By ROGER P. WODEHOUSE. Waltham, Mass.: Chronica Botanica Co., 1945. Pp. 245. Illustrated. $4.75.

This volume supplements a previous one by the same author (*Pollen Grains*, McGraw-Hill, 1935) on pollen grains of plants concerned with hayfever. Brief descriptions of the entire plants are given, and their geographical ranges and those of some of their near relatives are briefly noted. Some of the species are illustrated by means of line drawings, many of which are critical and helpful toward identification, others are too sketchy to be of any diagnostic value. A chapter on regional surveys, designed to furnish information on the hayfever plants occurring in various regions of the United States and on the period of the year during which the various species are of significance, presents available information in a condensed and usable form.—E. J. KRAUS.

Weeds of Lawn and Garden. By JOHN M. FOGG, JR. Philadelphia: University of Pennsylvania Press, 1945. Pp. 215. Illustrated. $2.50.

This readable handbook lists more than 175 species of plants which are likely to be encountered as garden and field weeds in eastern temperate North America. In addition to some material of general interest, each species is described in some detail, and the likely places where it may occur are indicated.

The line drawings accompanying the descriptive matter are well done, but it is doubtful whether the hopes of the author that the drawings will be of special aid in the recognition of specific weeds are likely to be realized. The illustrations are much more likely to be of value to the botanist than to the average gardener, whose experience scarcely enables him to recognize species by comparison with line drawings. The descriptive material is concise and pertinent.—E. J. KRAUS.

The Permeability of Living Cells. By S. C. BROOKS and MATILDA MOLDENHAUER BROOKS. Ann Arbor: Edwards Brothers, Inc., 1944. Pp. ix+396. Illustrated. $5.00.

This book is in a field in which the authors have already made many important contributions. It was published originally as one of the Protoplasma Monographs. The literature on the important theories of permeability and the main factors affecting it is summarized and critically evaluated. Seven chapters consider the permeability to water and the water and osmotic relations of plant and animal cells. Among the animal tissues considered are erythrocytes, eggs of marine invertebrates and of vertebrate animals, protozoa, muscle cells and nerves; also, the osmotic relations of bacteria, algae, fungi, and vascular plants are discussed. Five chapters deal with the permeability of living tissues to non-electrolytes, weak electrolytes and gases, strong electrolytes, and dyes, including a chapter on the oxidation-reduction dyes. The accumulation of ions by cells, especially plant cells, is considered at length.

There has long been need of a book of this kind, and biologists will welcome this critical résumé of the literature on permeability. The bibliography contains about 1400 references.—S. V. EATON.

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